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(54) Title: BACTERIAL ELASTIN BINDING PROTEIN, NUCLEIC ACID SEQUENCE ENCODING SAME AND DIAGNOSTIC AND THERAPEUTIC METHODS OF USE THEREOF

(57) Abstract

A microbial surface component recognizing adhesive matrix molecules (MSCRAMM) is disclosed which is characterized by its ability to bind to elastin, its inhibited activity in the presence of SDS and its enhanced activity in the presence of thiol reductants. More particularly, the MSCRAMM is bacterial in origin and is a member of the family of elastin binding proteins. In one embodiment, the MSCRAMM comprises a polypeptide having an amino acid sequence defined herein as SEQ ID NO:2, and extends to active fragments thereof. The role of MSCRAMMs in bacterial infection and its sequelae and related conditions is noted and the MSCRAMM may be prepared and used in diagnostic procedures and tests, including drug discovery assays, as well as in pharmaceutical compositions applicable for corresponding therapeutic methods. Both agonists and antagonists of the MSCRAMM are proposed and illustrated.

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BACTERIAL ELASTIN BINDING PROTEIN, NUCLEIC ACID SEQUENCE ENCODING SAME AND DIAGNOSTIC AND THERAPEUTIC METHODS OF USE THEREOF

GOVERNMENTAL SUPPORT

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TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to a microbial surface component recognizing adhesive matrix molecules (MSCRAMM) and to active polypeptide fragments thereof, and to nucleotide sequences encoding the protein and active polypeptide fragments thereof. More specifically, the invention relates to a protein on the surface of bacteria which binds a component of the extracellular matrix (ECM), which component is termed elastin, and to diagnostic and therapeutic methods which relate to this protein-protein interaction.

BACKGROUND OF THE INVENTION

The extracellular matrix (ECM) is a ubiquitous structure that contributes to architecture, elasticity, and regidity of virtually all vertebrate tissues and organs. Within the last several decades, additional biologic activities of the ECM have been identified. Distinct components of the ECM have been found to mediate one or several cellular events such as adhesion, proliferation, and regulation of gene expression (1-4). These cellular ECM interactions in turn direct many physiologic and pathologic processes including development, wound healing, and tumor cell metastasis (5-7). It is now known that cell surface ECM receptors are the key mediators of these biologic events. Most of these ECM receptors belong to a family of dimeric receptor complexes called integrins (8,9), although non-integrin ECM receptors have been identified (10). In addition to eukaryotic cells, various

pathogenic bacteria also interact specifically with the host ECM through cell surface ECM binding molecules. Cell surface ECM binding molecules of pathogenic bacteria belong to a group of proteins known collectively as adhesins or microbial surface components recognizing adhesive matrix molecules (MSCRAMMS), and are widely believed to play important roles in key steps of disease pathogenesis (11,12).

Among many important pathogenic bacteria, few are as efficient in developing multiple resistance to antibiotics and causing a wide spectrum of diseases as *Staphylococcus aureus (S. Aureus)*. *S. aureus* has been identified as one of the causative agents of diseases such as infective endocarditis, osteomyelitis, aortitis, pneumonia, and scalded skin syndrome (13-15). Furthermore, several strains of *S. aureus* have a propensity to extravasate into the circulation to cause bacteremia and subsequent formation of metastatic abscesses. These properties imply that *S. aureus* is capable of interacting with various components of the host expressed in respective target tissues. In agreement with this hypothesis, *S. aureus* has been found to associate with many host determinants including major ECM components such as collagen (16), fibronectin (17), laminin (18), proteoglycans (19), and elastin (20).

Most clinical isolates of *S. aureus* bind specifically to fibronectin, and mutant strains defective in fibronectin binding have decreased ability to colonize damaged heart valves in animal models of endocarditis (21). The *S. aureus*-collagen binding interaction has been implicated in osteomyelitis and septic arthritis by Switalski et al. (22), in which expression of the collagen adhesin has been found to be both necessary and sufficient for attachment of *S. aureus* to the type II collagen-rich cartilage. They also demonstrated in a murine experimental septic arthritis model that greater than 70% of animals injected with collagen adhesin-positive strains developed septic arthritis whereas less than 27% of animals challenged with isogenic mutant strains lacking the collagen adhesin developed clinical symptoms of the disease (23). As one of the main component of the basement membrane ECM, laminin has been proposed to play an important role in tumor cell metastasis. It has been shown that invasive *S.*

aureus interacts with the basement membrane component laminin, but, non-invasive *S. epidermidis* shows no binding (18). Taken together, these observations indicate that *S. aureus*-ECM interactions are playing critical roles in targeting host tissues for attachment, colonization, and invasion.

The primary physiologic role of elastin is to confer the property of reversible elasticity to tissues and organs (24). Elastin expression is highest in the lung, skin, and blood vessels, but the protein is widely expressed in mammalian hosts for *S. aureus*. Since elastin binding may be a mechanism for *S. aureus* to target elastin-rich tissues of the host, the cellular and biochemical properties of this interaction (20) were investigated in a previous study. *S. aureus* binding to elastin was found to be rapid, reversible, saturable, of high affinity (low nM), and ligand specific. Furthermore, a 25 kDa cell surface elastin binding protein named EbpS (for elastin binding protein of *Staphylococcus aureus*) was isolated, and EbpS has been proposed to mediate *S. aureus* binding to elastin-rich host ECM. EbpS is structurally distinct from the mammalian cell surface elastin binding protein, and the two elastin binding proteins recognize different regions in elastin. EbpS binds to a region in the N-terminal 30 kDa fragment of elastin, whereas the mammalian elastin receptor recognizes the hexapeptide sequence VGVAPG in the C-terminal half of elastin (25).

From the foregoing it is apparent that a greater understanding of EbpS would contribute to the effort to develop new diagnostic and therapeutic strategies against bacterial infection and the sequelae thereof, and that a concomitant need exists in the art for compositions and methods for such diagnostic and therapeutic intervention. Accordingly, it is toward the fulfillment of that need that the present invention responds.

SUMMARY OF THE INVENTION

In accordance with the present invention, a nucleotide sequence is provided that encodes a bacterial MSCRAMM, which is implicated in the attachment, colonization and invasion of host cells by the bacteria.

In a further embodiment, the invention provides the MSCRAMM protein, biologically active fragments thereof, and antibodies and binding partners thereto, which are useful in the diagnosis and treatment of bacterial infections. These reagents are particularly useful in treating infections of elastin-containing host tissues, for example lung, skin and blood vessels. Corresponding pharmaceutical compositions and therapeutic methods involving the MSCRAMMs of the present invention are contemplated, that may be directed to a broad range of diseases and other conditions, including by way of non-limiting illustration, such conditions as tumor cell metastasis, wound healing, infective endocarditis, osteomyelitis, aortitis, pneumonia and scalded skin syndrome.

One aspect of the present invention includes an MSCRAMM having the following characteristics:

a) it binds to elastin;

b) its activity is inhibited in the presence of SDS; and

c) it has enhanced activity in the presence of thiol reductants.

In a further aspect, the MSCRAMM has a predicted molecular weight of about 25 kDa, and a predicted PI of about 4.9. More particularly, the present MSCRAMM is isolated from *Staphylococcus aureus*.

Another aspect of the present invention extends to active polypeptide fragments of an MSCRAMM and related polypeptides which retain the property of binding elastin. In one embodiment, such a polypeptide comprises an amino acid sequence that corresponds to the elastin binding site of a MSCRAMM and consists of between 8 and 80 amino acids. In one such embodiment the polypeptide has the amino acid

sequence consisting of the N-terminal 59 amino acids of the MSCRAMM. In another embodiment the polypeptide consists of between 10 and 46 amino acids. In still another embodiment, the polypeptide consists of between 12 and 21 amino acids. In yet another embodiment, the polypeptide contains about 10 amino acids. In preferred embodiments the MSCRAMM has the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:2 comprising a conservative substitution thereof.

In another such embodiment the polypeptide corresponds to an elastin binding site of a MSCRAMM that comprises the amino acid sequence of SEQ ID NO:16 or SEQ ID NO:16 comprising a conservative substitution thereof (amino acids (AAs) 18-23 of SEQ ID NO:2: TNSHQD). In one particular embodiment the polypeptide has the amino acid sequence of SEQ ID NO:10 (AAs 1-78 of SEQ ID NO:2) or SEQ ID NO:10 comprising a conservative substitution thereof. In another particular embodiment the polypeptide has the amino acid sequence of SEQ ID NO:12 (AAs 1-34 of SEQ ID NO:2) or SEQ ID NO:12 comprising a conservative substitution thereof. In yet another particular embodiment the polypeptide comprises the amino acid sequence of SEQ ID NO:13 (AAs 14-34 of SEQ ID NO:2) or SEQ ID NO:13 comprising a conservative substitution thereof. In still another particular embodiment the polypeptide comprises the amino acid sequence of SEQ ID NO:14 (AAs 14-23 of SEQ ID NO:2) or SEQ ID NO:14 comprising a conservative substitution thereof. In still another particular embodiment the polypeptide consists of the amino acid sequence of SEQ ID NO:15 (AAs 14-59 of SEQ ID NO:2) or SEQ ID NO:15 comprising a conservative substitution thereof. In yet another particular embodiment the polypeptide of comprises the amino acid sequence of SEQ ID NO:18 (AAs 18-34 of SEQ ID NO:2) or SEQ ID NO:18 comprising a conservative substitution thereof. In a preferred embodiment the polypeptide further inhibits the binding of *S. aureus* to elastin.

The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a MSCRAMM or an active polypeptide

fragment thereof; preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene. In one particular embodiment the nucleic acid encodes a MSCRAMM having the DNA sequence shown in FIGURE 3 (SEQ ID NO:1).

In another such embodiment a nucleic acid encodes a polypeptide that binds elastin; consists of between 8 and 80 amino acids; and comprises an amino acid sequence that corresponds to the elastin binding site of a microbial surface component recognizing adhesive matrix molecules (MSCRAMM). In one particular embodiment the nucleic acid has the nucleic acid sequence of SEQ ID NO:9. In another particular embodiment, the nucleic acid has the nucleic acid sequence of SEQ ID NO:11.

The bacterial DNA sequences of the MSCRAMM of the present invention or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic libraries for the MSCRAMM. In particular, such probes may be degenerate probes based on SEQ ID NO:3. In addition, primers directed to the 5' and 3' sequences of the MSCRAMM, such as SEQ ID NOS: 4 and 5, may be used to amplify related sequences by PCR. For example, the probes may be prepared with a variety of known vectors, such as the phage λ vector. The present invention also includes the preparation of plasmids including such vectors, and the use of the DNA sequences to construct vectors expressing antisense RNA or ribozymes which would attack the mRNAs of any or all of the DNA sequences set forth in FIGURE 3 (SEQ ID NO:1, respectively). Correspondingly, the preparation of antisense RNA and ribozymes are included herein.

The present invention also includes MSCRAMM proteins having the activities noted herein, and that display the amino acid sequences set forth and described above and selected from SEQ ID NO:2.

In a further embodiment of the invention, the full DNA sequence of the recombinant DNA molecule or cloned gene so determined may be operatively linked to an expression control sequence which may be introduced into an appropriate host. The invention accordingly extends to unicellular hosts transformed with the cloned gene or recombinant DNA molecule comprising a DNA sequence encoding the present MSCRAM(s) active polypeptide fragments thereof and the polypeptides of the present invention. More particularly, a unicellular host is transformed with the complete DNA sequence determined from the sequences set forth above or a fragment of the DNA sequence, *e.g.* SEQ ID NO: 1, or SEQ ID NOs: 9 or 11.

According to other preferred features of certain preferred embodiments of the present invention, a recombinant expression system is provided to produce biologically active animal or human MSCRAMMs or the active polypeptide fragments thereof, or the polypeptides of the present invention.

The concept of the MSCRAMM contemplates that specific bacterial cell surface proteins exist for correspondingly specific components in host tissue, such as elastin and the like, as described earlier. Accordingly, the exact structure of each MSCRAMM will understandably vary so as to achieve this binding and activity specificity. It is this specificity and the direct involvement of the MSCRAMM in the chain of events leading to bacterial infection, that offers the promise of a broad spectrum of diagnostic and therapeutic utilities.

The present invention naturally contemplates several means for preparation and isolation of the MSCRAMM, including isolation from a natural source and/or as illustrated herein, through known recombinant techniques. The invention is accordingly intended to cover such preparations within its scope. Subsequent isolation of the MSCRAMM, active polypeptide fragments thereof and polypeptides of the present invention as illustrated herein, is also included as part of the invention. The isolation of the genomic DNA and amino acid sequences disclosed herein

facilitates the reproduction of the MSCRAMM by such recombinant techniques, and accordingly, the invention extends to expression vectors prepared from the disclosed DNA sequences for expression in host systems by recombinant DNA techniques, and to the resulting transformed hosts.

The invention includes an assay system for screening of potential drugs effective to modulate binding activity to target mammalian cells by interrupting or potentiating the binding of the MSCRAMM to host tissue. In one instance, the test drug could be administered to a cellular sample with the MSCRAMM that binds the host tissue, or an extract containing the MSCRAMM, to determine its effect upon the binding activity of the MSCRAMM to any chemical sample (including DNA), or to the test drug, by comparison with a control.

The assay system could more importantly be adapted to identify drugs or other entities that are capable of binding to the MSCRAMM, thereby inhibiting or potentiating infectivity. Such an assay would be useful in the development of drugs that would be specific against particular infections. For example, such drugs might be used to prevent infection, or to treat infection, as for example, in association with an antibiotic.

In yet a further embodiment, the invention contemplates antagonists of the activity of a MSCRAMM, in particular, an agent or molecule that inhibits MSCRAMM binding to elastin. In a specific embodiment, the antagonist can be a peptide having the sequence of a portion of an elastin-binding domain of a MSCRAMM.

One of the characteristics of the present MSCRAMM is that it binds to a 30 kDa N-terminal fragment of elastin, which is present in tissues which require elasticity, such as lungs, skin and blood vessels.

The diagnostic utility of the present invention extends to the use of the present MSCRAMM in assays to screen for bacterial infection.

The present invention likewise extends to the development of antibodies against the MSCRAMM(s) and active polypeptide fragments thereof and polypeptides of the invention, including naturally raised and recombinantly prepared antibodies. For example, the antibodies could be used to screen expression libraries to obtain the gene or genes that encode the MSCRAMM(s). Such antibodies could include both polyclonal and monoclonal antibodies prepared by known genetic techniques, as well as bi-specific (chimeric) antibodies, and antibodies including other functionalities thereby suiting them for additional diagnostic use conjunctive with their capability of modulating bacterial infectivity.

Thus, the MSCRAMMs active polypeptide fragements thereof, polypeptides of the invention, their analogs, cognates and/or mimics, and any antagonists or antibodies that may be raised thereto, are capable of use in connection with various diagnostic techniques, including immunoassays, such as a radioimmunoassay, using for example, an antibody to the MSCRAMM that has been labeled by either radioactive addition, or radioiodination.

In an immunoassay, a control quantity of the antagonists or antibodies thereto, or the like may be prepared and labeled with an enzyme, a specific binding partner and/or a radioactive element, and may then be introduced into a cellular sample. After the labeled material or its binding partner(s) has had an opportunity to react with sites within the sample, the resulting mass may be examined by known techniques, which may vary with the nature of the label attached.

In the instance where a radioactive label, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme,

detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

The present invention includes an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of the MSCRAMM, or to identify drugs or other agents that may mimic or block their activity. The system or test kit may comprise a labeled component prepared by one of the radioactive and/or enzymatic techniques discussed herein, coupling a label to the MSCRAMMs, their agonists and/or antagonists, and one or more additional immunochemical reagents, at least one of which is a free or immobilized ligand, capable either of binding with the labeled component, its binding partner, one of the components to be determined or their binding partner(s).

In a further embodiment, the present invention relates to certain therapeutic methods which would be based upon the activity of the MSCRAMM(s), its (or their) subunits, or active polypeptide fragments thereof, or polypeptides of the present invention or upon agents or other drugs determined to possess the same activity. A first therapeutic method is associated with the prevention of the manifestations of conditions causally related to or following from the binding activity of the MSCRAMM or its subunits, and comprises administering an agent capable of modulating the production and/or activity of the MSCRAMM or subunits thereof, either individually or in mixture with each other in an amount effective to prevent the development of those conditions in the host. For example, drugs or other binding partners to the MSCRAMM or proteins may be administered to inhibit bacterial infection. More particularly in the treatment of *S. aureus* infection.

More specifically, the therapeutic method generally referred to herein could include the method for the treatment of various pathologies or other cellular dysfunctions and derangements by the administration of pharmaceutical compositions that may

comprise effective inhibitors or enhancers of the MSCRAMM or its subunits, or other equally effective drugs developed for instance by a drug screening assay prepared and used in accordance with a further aspect of the present invention. For example, drugs or other binding partners to the MSCRAMM or proteins, as represented by SEQ ID NO:2, may be administered to inhibit bacterial infectivity.

In particular, the MSCRAMS active polypeptide fragments thereof and peptides of the present invention, their antibodies, agonists, antagonists, could be prepared in pharmaceutical formulations for administration in instances wherein antibiotic therapy is appropriate.

Accordingly, it is a principal object of the present invention to provide a MSCRAMM and its subunits in purified form that exhibits certain characteristics and activities associated with bacterial infectivity.

It is a further object of the present invention to provide antibodies to the MSCRAMM and its subunits, and methods for their preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting the presence of the MSCRAMM and its subunits in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

It is a further object of the present invention to provide a method and associated assay system for screening substances such as drugs, agents and the like, potentially effective in either mimicking the activity or combating the adverse effects of the MSCRAMM and/or its subunits in mammals.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the MSCRAMM or subunits thereof,

so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the MSCRAMM-containing bacteria or its subunits, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon the MSCRAMM, its subunits, active polypeptide fragments thereof, polypeptides of the present invention binding partner(s) to MSCRAMMs, or upon agents or drugs that control the production, or that mimic or antagonize the activities of the MSCRAMM.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing description which proceeds with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Southern analysis of genomic DNA from *S. aureus*. Genomic DNA isolated from *S. aureus* strain 12598 was digested with *Eco*R I (lanes A & C) or *Eco*R I/*Hind* III/*Hinc* II (lane D) and pEBPS-1 was digested with EcoR I (lane B). Samples were fractionated by 1% TAE-agarose electrophoresis and Southern blotted to nitrocellulose. The membranes were hybridized to a degenerate oligonucleotide (lanes A & B) or to the 2.6 kb *Hind* III/*Hinc* II insert of pKS-2.6 (lanes C & D). Sizes of the hybridized fragments were determined from the migration pattern of *Hind* III-digested λ DNA markers.

Figure 2: Physical map of the pKS-2.6 insert. Sites recognized by various restriction endonucleases are indicated. Location and direction of the *ebpS* open reading frame is shown by the hatched box and arrow, respectively.

Figure 3: Primary sequence of *ebpS*. Nucleotide and predicted amino acid sequences are numbered starting at the first nucleotide of the open reading frame and translation initiation codon, respectively. The putative -35 and -10 hexamers, and ribosomal binding site are indicated. The experimentally determined N-terminal sequence of cell surface EbpS is shown in bold letters, and the experimentally determined amino acid sequences of EbpS are underlined. The N-terminal amino acids of the recombinant construct were sequenced from both full length rEbpS and N-terminal fragment of CNBr-cleaved rEbpS. The in-frame termination codon is indicted by an asterisk.

Figure 4: Expression of *ebpS* gene in *E. coli*. The *ebpS* open reading frame was PCR-amplified and expressed in *E. coli* as a fusion protein with polyhistidine residues attached to the N-terminus. rEbpS purified from three different positive clones by Ni++-NTA affinity chromatography was fractionated by 10% SDS-PAGE and stained with Coomassie Brilliant Blue R-250 (lanes B-D). Migration pattern of the size standard is shown in lane A.

Figure 5: rEbpS binds specifically to immobilized elastin peptides. Approximately 10^6 cpm of radiolabeled rEbpS was incubated with 1 ml of the elastin peptide affinity resin in the absence (lane C) or presence (lane D) of 2 mg of unlabeled elastin peptides for 2 h at room temperature in 1.5 ml of binding buffer. After thorough washing, bound proteins were eluted with 1% SDS buffer and analyzed by 10% SDS-PAGE and autoradiography. The starting material contained a 40 kDa degradation product in addition to the intact 45 kDa rEbps (lane B). Migration of ^{14}C-labeled size standards is shown in lane A.

Figure 6: rEbpS specifically inhibits *S. aureus* binding to radiolabeled elastin. Radioiodinated elastin (20 ng) was incubated with 2 x 10^8 live *S. aureus* cells in the absence or presence of 1.0, 2.0, 5.9, 9.8, or 19.6 μM of unlabeled rEbpS or 26 μM of mouse DHFR for 1 h at room temperature in 200 μl of TSB. The cells were pelleted by centrifugation, and the supernatant was discarded. Pellets were resuspended in 1 ml of TSB, transferred to new tubes, and washed two more time with TSB. Radioactivity associated with cells was measured using a gamma counter. Results are presented as mean % binding ± standard deviation of triplicate determinations.

Figure 7: Cell surface labeled EbpS binds to an antibody against rEbps. Cell surface labeled extracts (10^7 cpm) prepared by IODOGEN radioiodination and subsequent lysostaphin digestion (lane A) were pre-absorbed to 3 ml of pig IgG-Affi-Gel 10 to remove protein from the starting material. Starting material devoid of surface labeled protein A (lane B) was incubated with 1 ml of the anti-rEbpS IgG affinity resin in the absence (lane C) or presence (lane D) of 2 mg unlabeled rEbpS in 2 ml of binding buffer for 2 h at room temperature. After washing with buffer until radioactivity of the flow through reached background levels, bound surface proteins were eluted with 1% SDS and analyzed by 15% SDS-PAGE and autoradiography. The band intensity of proteins (25 & 35 kDa) that associated with the affinity resin was scanned to quantify binding.

Figure 8: Fab fragments of anti-rEbpS IgG inhibit *S. aureus* binding to elastin. Radiolabeled elastin was incubated with live *S. aureus* cells in the absence or presence of 6, 10, 20, 50, and 100μg of immune rEbpS IgG Fab or 20 and 100 μg of pre-immune Fab fragments for 1 h at room temperature in 2 ml of binding buffer, and binding was quantified as previously described. Data are presented as mean % binding ± standard deviation of triplicate measurements.

Figure 9: The elastin binding site in EbpS is contained within residues 14-59. Elastin binding properties of various EbpS fragments and recombinant constructs (described

in Example 2) were assessed by their capacity to specifically bind to tropoelastin. Inactivity of the amino terminal synthetic peptide was determined by an inability to inhibit binding. Residues 14-59 (shaded area) are common to all fragments with elastin binding activity.

Figure 10: Expression of recombinant EbpS proteins. Recombinant EbpS proteins were purified by Ni-NTA affinity chromatography, fractionated by 15% SDS-PAGE, and stained with Coomassie Brilliant Blue R-250 (a) or transferred to nitrocellulose membranes and reacted with anti-rEbpS IgG (b) or anti-rEbpS IgG that had been pre-absorbed to trEbpS-2 (c). Lane A: ovalbumin, lane B: rEbpS, lane C: trEbpS-1, lane D: trEbpS-2, and lane E: lysozyme. Molecular masses of the recombinant proteins were approximated from the migration pattern of ovalbumin, lysozyme, and pre-stained size standards.

Figure 11: Recombinant trEbpS-1 and trEbpS-2 bind to elastin. Tropolastin (3 μg) that was fractionated by 10% SDS-PAGE and Western blotted to nitrocellulose membranes was reacted with 5 μM biotinylated trEbpS-1 (lanes A and B) or trEbpS-2 (lanes C and D) in the absence (lanes A and C) or presence (lanes B and D) of 3 mg/ml elastin peptides. Binding of truncated EbpS proteins was visualized by subsequent incubation with avidin-horse radish peroxidase and 4-chloro-naphthol.

Figure 12: Truncated recombinant EbpS proteins inhibit binding of *S. aureus* cells to radiolabeled elastin. Live *S. aureus* cells ($2x10^8$) were incubated with radioiodinated elastin (10 ng) in the absence or presence of increasing concentrations of rEbpS, trEbpS-1, or trEbpS-2 for 1 hour at room temperature in 200 μl of TSB. The assay was terminated by centrifugation, and cell pellets were washed twice with 1 ml of TSB. Extent of binding was quantified by measuring radioactivity associated with the pellets. Results are presented as mean relative % binding±SD of triplicate determinations, with measurements obtained in the absence of recombinant EbpS proteins defined as 100%.

Figure 13: The inhibitory effect of anti-rEbpS antibody is neutralized by pre-absorption with trEbpS-2. Fab fragments from the original (control) and anti-rEbpS IgGs that had been pre-absorbed with trEbpS-2 (trEbpS-2 negative) were prepared by papain digestion. Increasing concentrations of Fab fragments were incubated with live *S. aureus* cells and radiolabeled elastin as previously described. Data are shown as mean relative % binding±SD from triplicate determinations.

Figure 14: Elastin binding site defined by peptide inhibition studies. Seven overlapping synthetic peptides spanning residues 14-36 were tested for their ability to inhibit binding of *S. aureus* to tropoelastin. Inhibition activity, qualitatively scored as + or -, is indicated next to the peptide number. Some amino acids in peptides P2 and P7 were substituted as indicated. The shaded box indicates the predicted active sequence required for elastin binding.

Figure 15: Peptide P1 spanning EbpS residues 18-34 specifically inhibits binding of *S. aureus* to radioiodinated elastin. Increasing concentrations of the P1 and P2 peptides were incubated with *S. aureus* cells and radiolabeled elastin for 1 hour at room temperature. Binding was quantified as previously described. Results are presented as mean relative % binding±SD (n=3).

Figure 16: A synthetic peptide corresponding to EbpS residues 14-23 specifically inhibits staphylococcal elastin binding. Overlapping synthetic 10mers corresponding to residues 14-23 (P3), 21-30 (P4), and 27-36 (P5) were generated as described. *S. aureus* cells were incubated with labeled elastin in the absence or presence of 0.5, 1.0, or 2.0 mg/ml of P3-P5 peptides. Binding assays were processed as described previously. Error bars represent SD calculated from triplicate determinations.

DETAILED DESCRIPTION

Early studies on the binding of *Staphylococcus aureus* were described in Park et al, *J. Biol. Chem.* **266**:23399-23406 (1991), which is hereby incorporated by reference in its entirety. MSCRAMM-mediated adherence of microorganisms to host tissues is reviewed in Patti et al, *Annu. Rev. Microbiol.* **48**:585-617 (1994), which is also hereby incorporated by reference in its entirety.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

The terms "MSCRAMM," "bacterial cell surface protein," "elastin binding protein (ebpS)," and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application and claims refer to proteinaceous material including single or multiple proteins, and extends to those proteins having the amino acid sequence data described herein and presented in FIGURE 3 (SEQ ID NO:2), and the profile of activities set forth herein and in the Claims. Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such

as those obtained through mutations in hosts that are producers of the complex or its named subunits. Also, the terms "MSCRAMM," "bacterial cell surface protein" and "elastin binding protein (ebpS)" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

As used herein, an "active polypeptide fragment" of a MSCRAMM is a polypeptide fragment of an MSCRAMM that binds to elastin. A "polypeptide of the present invention" is a polypeptide that comprises an amino acid sequence that corresponds to the elastin binding site of an MSCRAMM. Such polypeptides consist of between 8 and 80 amino acids.

The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired fuctional property of immunoglobulin-binding is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.*, **243**:3552-59 (1969), abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		AMINO ACID
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine

I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
C	Cys	cysteine

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

The term "polypeptide" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other the bonds, *e.g.*, ester, ether, etc.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to hybridize therewith and thereby form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

It should be appreciated that also within the scope of the present invention are DNA sequences encoding MSCRAMM which code for an MSCRAMM having the same amino acid sequence as SEQ ID NO:2, but which are degenerate to SEQ ID NO:2. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid. It is well known in the art that the following codons can be used interchangeably to code for each specific amino acid:

Phenylalanine (Phe or F)	UUU or UUC
Leucine (Leu or L)	UUA or UUG or CUU or CUC or CUA or CUG
Isoleucine (Ile or I)	AUU or AUC or AUA
Methionine (Met or M)	AUG
Valine (Val or V)	GUU or GUC of GUA or GUG
Serine (Ser or S)	UCU or UCC or UCA or UCG or AGU or AGC
Proline (Pro or P)	CCU or CCC or CCA or CCG
Threonine (Thr or T)	ACU or ACC or ACA or ACG
Alanine (Ala or A)	GCU or GCG or GCA or GCG
Tyrosine (Tyr or Y)	UAU or UAC
Histidine (His or H)	CAU or CAC
Glutamine (Gln or Q)	CAA or CAG
Asparagine (Asn or N)	AAU or AAC
Lysine (Lys or K)	AAA or AAG
Aspartic Acid (Asp or D)	GAU or GAC
Glutamic Acid (Glu or E)	GAA or GAG
Cysteine (Cys or C)	UGU or UGC
Arginine (Arg or R)	CGU or CGC or CGA or CGG or AGA or AGG
Glycine (Gly or G)	GGU or GGC or GGA or GGG
Tryptophan (Trp or W)	UGG
Termination codon	UAA (ochre) or UAG (amber) or UGA (opal)

It should be understood that the codons specified above are for RNA sequences. The corresponding codons for DNA have a T substituted for U.

Mutations can be made in SEQ ID NO:1 such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of

amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting polypeptide. Accordingly, such conservative changes are defined herein as a "conservative substitution".

The following is one example of various groupings of amino acids:

Amino acids with nonpolar R groups

Alanine
Valine
Leucine
Isoleucine
Proline
Phenylalanine
Tryptophan
Methionine

Amino acids with uncharged polar R groups

Glycine
Serine
Threonine
Cysteine

Tyrosine

Asparagine

Glutamine

Amino acids with charged polar R groups (negatively charged at ph 6.0)

Aspartic acid

Glutamic acid

Basic amino acids (positively charged at pH 6.0)

Lysine

Arginine

Histidine (at pH 6.0)

Another grouping may be those amino acids with phenyl groups:

Phenylalanine

Tryptophan

Tyrosine

Another grouping may be according to molecular weight (i.e., size of R groups):

Glycine	75
Alanine	89
Serine	105
Proline	115
Valine	117
Threonine	119
Cysteine	121

Leucine	131
Isoleucine	131
Asparagine	132
Aspartic acid	133
Glutamine	146
Lysine	146
Glutamic acid	147
Methionine	149
Histidine (at pH 6.0)	155
Phenylalanine	165
Arginine	174
Tyrosine	181
Tryptophan	204

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH_2 can be maintained.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced into a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β-turns in the protein's structure.

Two amino acid sequences are "substantially homologous" when at least about 70% of the amino acid residues (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and $F(ab')_2$ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from $F(ab')_2$ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in the S phase activity of a target cellular mass, or other feature of pathology such as for example, elevated blood pressure, fever or white cell count as may attend its presence and activity.

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined T_m with washes of higher stringency, if desired.

In its primary aspect, the present invention concerns the identification of a bacterial MSCRAMM which binds a component of the extracellular matrix, mediating the attachment, colonization and/or invasion of the bacterial into a host tissue.

In a particular embodiment, the present invention relates to all members of the herein disclosed elastin binding proteins.

As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a MSCRAMM, or a fragment thereof, that possesses a predicted molecular weight of about 25 kD and an amino acid sequence set forth in FIGURE 3 (SEQ ID NO:2); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 25 kD has a nucleotide sequence or is complementary to a DNA sequence shown in FIGURE 2 (SEQ ID NO:1).

Initial steps for purifying an MSCRAMM or active polypeptide fragment thereof of the present invention include salting in or salting out, such as in ammonium sulfate fractionations; solvent exclusion fractionations, *e.g.*, an ethanol precipitation; detergent extractions to free membrane bound proteins using such detergents as TRITON X-100, TWEEN-20 etc.; or high salt extractions. Solubilization of proteins or polypeptides may also be achieved using aprotic solvents such as dimethyl sulfoxide and hexamethylphosphoramide. In addition, high speed ultracentrifugation may be used either alone or in conjunction with other extraction techniques.

Generally good secondary isolation or purification steps include solid phase absorption using calcium phosphate gel or hydroxyapatite; or solid phase binding. Solid phase binding may be performed through ionic bonding, with either an anion exchanger, such as diethylaminoethyl (DEAE), or diethyl [2-hydroxypropyl] aminoethyl (QAE) SEPHADEX or cellulose; or with a cation exchanger such as carboxymethyl (CM) or sulfopropyl (SP) SEPHADEX or cellulose. Alternative means of solid phase binding includes the exploitation of hydrophobic interactions *e.g.*, the using of a solid support such as phenylSepharose and a high salt buffer; affinity-binding, using, *e.g.*, elastin on an activated support; immuno-binding, using *e.g.*, an antibody to an MSCRAMM or active polypeptide fragment thereof bound to an activated support; as well as other solid phase supports including those that contain specific dyes or lectins etc. A further solid phase support technique that is often used at the end of the purification procedure relies on size exclusion, such as SEPHADEX

and SEPHAROSE gels, or pressurized or centrifugal membrane techniques, using size exclusion membrane filters.

Solid phase support separations are generally performed batch-wise with low-speed centrifugations or by column chromatography. High performance liquid chromatography (HPLC), including such related techniques as FPLC, is presently the most common means of performing liquid chromatography. Size exclusion techniques may also be accomplished with the aid of low speed centrifugation.

In addition size permeation techniques such as gel electrophoretic techniques may be employed. These techniques are generally performed in tubes, slabs or by capillary electrophoresis.

Almost all steps involving protein purification employ a buffered solution. Unless otherwise specified, generally 25-100 μM salt concentrations of buffer salts are used. Low concentration buffers generally imply 5-25 μM concentrations. High concentration buffers generally imply concentrations of the buffering agent of between 0.1-2M concentrations. Typical buffers can be purchased from most biochemical catalogues and include the classical buffers such as Tris, pyrophosphate, monophosphate and diphosphate and the Good buffers [Good, N.E., *et al.*, *Biochemistry*, **5**:467 (1966); Good, N.E. and Izawa, S., *Meth. Enzymol.*, **24B**:53 (1972); and Fergunson, W.J. and Good, N. E., *Anal. Biochem.*, **104**:300 (1980] such as Mes, Hepes, Mops, tricine and Ches.

Materials to perform all of these techniques are available from a variety of sources such as Sigma Chemical Company in St. Louis, Missouri.

The possibilities both diagnostic and therapeutic that are raised by the existence of the MSCRAMM, derive from the fact that the factors appear to participate in direct and causal protein-protein interaction between the MSCRAMM, and those factors that are

in the extracellular matrix to which the MSCRAMM binds. As suggested earlier and elaborated further on herein, the present invention contemplates pharmaceutical intervention in the cascade of reactions in which the MSCRAMM is implicated, to modulate the activity initiated by the MSCRAMM.

Thus, in instances where it is desired to reduce or inhibit the deleterious effects resulting from interaction of the MSCRAMM with a particular stimulus or factor, an appropriate inhibitor of the MSCRAMM could be introduced to block the interaction of the MSCRAMM with those factors causally connected with bacterial adhesion thereby.

As discussed earlier, the MSCRAMM or their binding partners or other ligands or agents exhibiting either mimicry or antagonism to the MSCRAMM or control over their production, may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated with specific bacterial infection for the treatment thereof. A variety of administrative techniques may be utilized, among them parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of the MSCRAMM or their subunits may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of the MSCRAMM and/or their subunits may possess certain diagnostic applications and may for example, be utilized for the purpose of detecting and/or measuring conditions such as bacterial infection or the like. For example, the MSCRAMM or its subunits may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Likewise, small molecules that mimic

or antagonize the activity(ies) of the MSCRAMM of the invention may be discovered or synthesized, and may be used in diagnostic and/or therapeutic protocols.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against MSCRAMM peptides can be screened for various properties; i.e., isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the binding activity of the MSCRAMM or its subunits, in particular the binding activity of the first 59 amino acids of the molecule to the amino terminal portion of elastin. Such monoclonals can be readily identified in binding activity assays such as ELISA or WESTERN BLOT. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant MSCRAMM is possible.

Preferably, the anti-MSCRAMM antibody used in the diagnostic methods of this invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-MSCRAMM antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$, or F(v) portions of whole antibody molecules.

As suggested earlier, the diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an antagonist to a MSCRAMM/protein, such as an anti-MSCRAMM

antibody, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the anti-MSCRAMM antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions of whole antibody molecules. As previously discussed, patients capable of benefiting from this method include those suffering from bacterial infections associated with cancer, a pre-cancerous lesion, a viral infection or other like pathological derangement. Methods for isolating the MSCRAMM and inducing anti-MSCRAMM antibodies and for determining and optimizing the ability of anti-MSCRAMM antibodies to assist in the examination of the target cells are all well-known in the art.

Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or $F(ab')_2$ portions of useful antibody molecules, can be prepared using the hybridoma technology described in *Antibodies - A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated herein by reference. Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with an elastin-binding portion thereof.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact with the present MSCRAMM and their ability to inhibit specified MSCRAMM activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the

hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., *Virol.* **8**:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal anti-MSCRAMM antibodies are also well-known in the art. See Niman et al., *Proc. Natl. Acad. Sci. USA*, **80**:4949-4953 (1983). Typically, the present MSCRAMM or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen in the before described procedure for producing anti-MSCRAMM monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with the MSCRAMM peptide analog and the present MSCRAMM.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of a MSCRAMM, polypeptide analog thereof or fragment thereof, as described herein as an active ingredient. In a preferred embodiment, the composition comprises an antigen capable of modulating the specific binding of the present MSCRAMM to a target cell.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions,

however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be

administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of MSCRAMM binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

The therapeutic compositions may further include an effective amount of the MSCRAMM/MSCRAMM antagonist or analog thereof, and one or more of the following active ingredients: an antibiotic, a steroid. Exemplary formulations are given below:

Formulations

Intravenous Formulation I

Ingredient	mg/ml
cefotaxime	250.0
MSCRAMM	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation II

Ingredient	mg/ml
ampicillin	250.0
MSCRAMM	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation III

Ingredient	mg/ml
gentamicin (charged as sulfate)	40.0
MSCRAMM	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation IV

Ingredient	mg/ml
MSCRAMM	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation V

Ingredient	mg/ml
MSCRAMM antagonist	5.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

As used herein, "pg" means picogram, "ng" means nanogram, "ug" or "μg" mean microgram, "mg" means milligram, "ul" or "μl" mean microliter, "ml" means milliliter, "l" means liter.

The polypeptides of the present invention can be can be chemically synthesized. The synthetic polypeptides are prepared using the well known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N^{α}-amino protected N^{α}-t-butyloxycarbonyl) amino acid resin with the standard deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield [*J. Am. Chem. Soc.*, **85**:2149-2154 (1963)], or the base-labile N^{α}-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han [*J. Org. Chem.*, **37**:3403-3409 (1972)]. Both Fmoc and Boc N^{α}-amino protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art. In addition, the method of the invention can be used with other N^{α}-protecting groups that are familiar to those skilled in this art. Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, IL; Fields and Noble, 1990, Int. J. Pept. Protein Res. 35:161-214, or using automated synthesizers, such as sold by ABS. Thus, polypeptides of the invention may comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (*e.g.*, β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc.) to convey special properties. Synthetic amino acids include ornithine for lysine, fluorophenylalanine for phenylalanine, and norleucine for leucine or isoleucine. Additionally, by assigning specific amino acids at specific coupling steps, α-helices, β turns, β sheets, γ-turns, and cyclic peptides can be generated.

In a further embodiment, subunits of peptides that confer useful chemical and structural properties will be chosen. For example, peptides comprising D-amino acids will be resistant to L-amino acid-specific proteases *in vivo*. In addition, the present invention envisions preparing peptides that have more well defined structural properties, and the use of peptidomimetics, and peptidomimetic bonds, such as ester bonds, to prepare peptides with novel properties. In another embodiment, a peptide may be generated that incorporates a reduced peptide bond, i.e., R_1-**CH_2-NH-**R_2, where R_1 and R_2 are amino acid residues or sequences. A reduced peptide bond may be introduced as a dipeptide subunit. Such a molecule would be resistant to peptide bond hydrolysis, *e.g.*, protease activity. Such peptides would provide ligands with unique function and activity, such as extended half-lives *in vivo* due to resistance to metabolic breakdown, or protease activity. Furthermore, it is well known that in certain systems constrained peptides show enhanced functional activity [Hruby, *Life Sciences*, **31**:189-199 (1982)]; [Hruby *et al.*, *Biochem J.*, **268**:249-262 (1990)]; the present invention provides a method to produce a constrained peptide that incorporates random sequences at all other positions.

Non-classical amino acids that induce conformational constraints:

The following non-classical amino acids may be incorporated in the peptide in order to introduce particular conformational motifs: 1,2,3,4-tetrahydroisoquinoline-3-carboxylate [Kazmierski *et al.*, *J. Am. Chem. Soc.*, **113**:2275-2283 (1991)]; (2S,3S)-methyl-phenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine [Kazmierski and Hruby, *Tetrahedron Lett.*, (1991)]; 2-aminotetrahydronaphthalene-2-carboxylic acid [Landis, *Ph.D. Thesis, University of Arizona*, (1989)]; hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate [Miyake *et al.*, *J. Takeda Res. Labs.*, **43**:53-76 (1989)]; β-carboline (D and L) [Kazmierski, *Ph.D. Thesis, University of Arizona*, (1988)]; HIC (histidine isoquinoline carboxylic acid) [Zechel *et al.*, *Int. J. Pep. Protein Res.*, 43 (1991)]; and HIC (histidine cyclic urea) (Dharanipragada).

The following amino acid analogs and peptidomimetics may be incorporated into a peptide to induce or favor specific secondary structures: LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a β-turn inducing dipeptide analog (Kemp *et al., J. Org. Chem.*, **50**:5834-5838 (1985)]; β-sheet inducing analogs [Kemp *et al., Tetrahedron Lett.*, **29**:5081-5082 (1988)]; β-turn inducing analogs [Kemp *et al., Tetrahedron Lett.*, **29**:5057-5060 (1988)]; ∝-helix inducing analogs [Kemp *et al., Tetrahedron Lett.*, **29**:4935-4938 (1988)]; γ-turn inducing analogs [Kemp *et al., J. Org. Chem.*, **54**:109:115 (1989)]; and analogs provided by the following references: Nagai and Sato, *Tetrahedron Lett.*, **26**:647-650 (1985); DiMaio *et al., J. Chem. Soc. Perkin Trans.*, p. 1687 (1989); also a Gly-Ala turn analog [Kahn *et al., Tetrahedron Lett.*, **30**:2317 (1989)]; amide bond isostere [Jones *et al., Tetrahedron Lett.*, **29**:3853-3856 (1988)]; tretrazol [Zabrocki *et al., J. Am. Chem. Soc.*, **110**:5875-5880 (1988)]; DTC [Samanen *et al., Int. J. Protein Pep. Res.*, **35**:501:509 (1990)]; and analogs taught in Olson *et al., J. Am. Chem. Sci.*, **112**:323-333 (1990) and Garvey *et al., J. Org. Chem.*, **56**:436 (1990). Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Patent No. 5,440,013, issued August 8, 1995 to Kahn.

Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host.

Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for

example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col El, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAS, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast α-mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, Rl.l, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly with regard to potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

It is further intended that MSCRAMM analogs may be prepared from nucleotide sequences of the protein complex/subunit derived within the scope of the present invention. Analogs, such as fragments, may be produced, for example, by pepsin digestion of bacterial material. Other analogs, such as muteins, can be produced by standard site-directed mutagenesis of MSCRAMM coding sequences. Analogs

exhibiting "elastin binding activity" such as small molecules, whether functioning as promoters or inhibitors, may be identified by known *in vivo* and/or *in vitro* assays.

As mentioned above, a DNA sequence encoding MSCRAMM can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the MSCRAMM amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature*, **292**:756 (1981); Nambair et al., *Science*, **223**:1299 (1984); Jay et al., *J. Biol. Chem.*, *259*:6311 (1984).

Synthetic DNA sequences allow convenient construction of genes which will express MSCRAMM analogs or "muteins". Alternatively, DNA encoding muteins can be made by site-directed mutagenesis of native MSCRAMM genes or cDNAs, and muteins can be made directly using conventional polypeptide synthesis.

A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael C. Griffith, Peter G. Schultz, *Science*, **244**:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

The present invention extends to the preparation of antisense oligonucleotides and ribozymes that may be used to interfere with the expression of the MSCRAMM at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. (See Weintraub, 1990; Marcus-Sekura,

1988.) In the cell, they hybridize to that mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into MSCRAMM-producing cells. Antisense methods have been used to inhibit the expression of many genes *in vitro* (Marcus-Sekura, 1988; Hambor et al., 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it [Cech, *J. Am. Med. Assoc.*, **260**:3030 (1988)]. Because they are sequence-specific, only mRNAs with particular sequences are inactivated.

Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. (Hasselhoff and Gerlach, 1988) *Tetrahymena*-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are preferable to *Tetrahymena*-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs for MSCRAMM and their ligands.

The present invention also relates to a variety of diagnostic applications, including methods for detecting the presence of stimuli such as the earlier referenced polypeptide ligands, by reference to their ability to elicit the activities which are mediated by the present MSCRAMM. As mentioned earlier, the MSCRAMM can be used to produce antibodies to itself by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the presence of particular MSCRAMM activity in suspect target tissues.

As described in detail above, antibody(ies) to the MSCRAMM can be produced and isolated by standard methods including the well known hybridoma techniques. For convenience, the antibody(ies) to the MSCRAMM will be referred to herein as Ab_1 and antibody(ies) raised in another species as Ab_2.

The presence of MSCRAMM in cells can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful utilize either the MSCRAMM labeled with a detectable label, antibody Ab_1 labeled with a detectable label, or antibody Ab_2 labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the particle is labeled, and "M" stands for the MSCRAMM:

A. $M^* + Ab_1 = M^*Ab_1$

B. $M + Ab^* = MAb_1^*$

C. $M + Ab_1 + Ab_2^* = MAb_1Ab_2^*$

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody," or "DASP" procedure.

In each instance, the MSCRAMM forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.

It will be seen from the above, that a characteristic property of Ab_2 is that it will react with Ab_1. This is because Ab_1 raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab_2. For example, Ab_2 may be raised in goats using rabbit antibodies as antigens. Ab_2 therefore would be anti-rabbit antibody raised in goats. For purposes of this description and claims, Ab_1 will be referred to as a primary or anti-MSCRAMM antibody, and Ab_2 will be referred to as a secondary or anti-Ab_1 antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

The MSCRAMM or its binding partner(s) can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the

like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, ß-glucuronidase, ß-D-glucosidase, ß-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in accordance with the present invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

Accordingly, a purified quantity of the MSCRAMM may be radiolabeled and combined, for example, with antibodies or other inhibitors thereto, after which binding studies would be carried out. Solutions would then be prepared that contain various quantities of labeled and unlabeled uncombined MSCRAMM, and cell samples would then be inoculated and thereafter incubated. The resulting cell monolayers are then washed, solubilized and then counted in a gamma counter for a length of time sufficient to yield a standard error of <5%. These data are then subjected to Scatchard analysis after which observations and conclusions regarding material activity can be drawn. While the foregoing is exemplary, it illustrates the manner in which a receptor assay may be performed and utilized, in the instance where the cellular binding ability of the assayed material may serve as a distinguishing characteristic.

An assay useful and contemplated in accordance with the present invention is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is a plasmid

that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the response element to the particular receptor is inserted. If the compound under test is an agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves are obtained and compared to those of known ligands. The foregoing protocol is described in detail in U.S. Patent No. 4,981,784 and PCT International Publication No. WO 88/03168, for which purpose the artisan is referred.

In a further embodiment of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of predetermined MSCRAMM activity or predetermined elastin binding activity capability in suspected target cells. In accordance with the testing techniques discussed above, one class of such kits will contain at least the labeled MSCRAMM or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., "competitive," "sandwich," "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Accordingly, a test kit may be prepared for the demonstration of the presence or capability of cells for predetermined elastin binding activity, comprising:

(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of the present MSCRAMM factor or a specific binding partner thereto, to a detectable label;

(b) other reagents; and

(c) directions for use of said kit.

More specifically, the diagnostic test kit may comprise:

(a) a known amount of the MSCRAMM as described above (or a binding partner) generally bound to a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;

(b) if necessary, other reagents; and

(c) directions for use of said test kit.

In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive," "sandwich," "double antibody," etc.), and comprises:

(a) a labeled component which has been obtained by coupling the MSCRAMM to a detectable label;

(b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:

(i) a ligand capable of binding with the labeled component (a);

(ii) a ligand capable of binding with a binding partner of the labeled component (a);

(iii) a ligand capable of binding with at least one of the component(s) to be determined; and

(iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and

(c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the MSCRAMM and a specific binding partner thereto.

In accordance with the above, an assay system for screening potential drugs effective to modulate the activity of the MSCRAMM may be prepared. The MSCRAMM may be introduced into a test system, and the prospective drug may also be introduced into

the resulting cell culture, and the culture thereafter examined to observe any changes in the MSCRAMM activity of the cells, due either to the addition of the prospective drug alone, or due to the effect of added quantities of the known MSCRAMM.

PRELIMINARY CONSIDERATIONS

The interaction between staphylococci and components of the extracellular matrix mediates attachment to host tissues and organs, and is an important mechanism leading to colonization, invasion, and formation of metastatic abscesses. A specific binding interaction has been demonstrated between *Staphylococcus aureus* and elastin, one of the major protein components of the extracellular matrix. Available evidence suggests that this association is mediated by a cell surface 25 kDa staphylococcal elastin binding protein (EbpS). To study the molecular structure and function of EbpS, the gene encoding EbpS was cloned, sequenced, and expressed in *E. coli*. DNA sequence data indicate that the ebpS open reading frame consists of 606 bp, and encodes a novel polypeptide of 202 amino acids. EbpS protein has a predicted molecular mass of 23,345 daltons and pI of 4.9. EbpS was expressed in *E. coli* as a fusion protein with polyhistidine residues attached to the N-terminus. A polyclonal antibody raised against recombinant EbpS interacted specifically with the 25 kDa cell surface EbpS and inhibited staphylococcal elastin binding. Furthermore, recombinant EbpS bound specifically to immobilized elastin and inhibited binding of *Staphylococcus aureus* to elastin. A degradation product of recombinant EbpS lacking the first 59 amino acids of the molecule and a C-terminal fragment of CNBr-cleaved recombinant EbpS, however, did not interact with elastin. These results strongly suggest that EbpS is the cell surface molecule mediating binding of *Staphylococcus aureus* to elastin. The finding that some constructs of recombinant EbpS do not interact with elastin suggests that the elastin binding site in EbpS is contained in the first 59 amino acids of the molecule.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

Materials and Methods

Materials

Restriction endonucleases, calf intestinal phosphatase, T4 DNA ligase, T4 polynucleotide kinase, isopropyl-ß-D-galactoside (X-gal), Wizard Miniprep plasmid purification kit, and Hind III-digested λ DNA markers were purchased from Promega (Madison, WI). DNase-free RNase was obtained from Boehringer Mannheim (Indianapolis IN). Luria-Bertani (LB) medium and LB agar-medium capsules were from BIO 101 (La Jolla, CA). Tryptic soy broth (TSB) was obtained from Remel (Lenexa, KS). High melting point agarose was purchased from Fisher (St. Louis, MO) and SeaPlaque GTG Agarose (low melting) was obtained from FMC BioProducts (Rockland, ME). $Na^{125}I$, γ-^{32}P-ATP, and α-^{32}P-CTP were from ICN (Costa Mesa, CA). Papain and protein A immobilized to cross linked agarose, and IODOGEN were purchased from Pierce (Rockford, IL). Rapid-hyb buffer and rediprime DNA labeling system were obtained from Amersham (Arlington Heights, IL). Chroma Spin-10 columns were purchased from Clontech (Palo Alto, CA). QIAexpress vector kit type IV and the midi-prep plasmid purification kit were obtained from Qiagen (Chatsworth, CA). Nitrocellulose membrane and blotting paper were from Schleicher & Schuell (Keene, NH). Affi-Gel-10 affinity support was from Bio-Rad (Melville, NY). All other materials were purchased from Sigma Chemical (St. Louis, MO).

Bacterial, plasmids, and culture conditions

S. aureus strain 12598 (Cowan) was purchased from the American Type Culture Collection (Rockville, MD). *E. coli* strains DH5α competent cells (MAX Efficiency) and M15 (pREP4) were from Gibco BRL (Gaithersburg, MD) and Qiagen, respectively. M15 cells contain the plasmid pREP4 which constitutively expresses the lac repressor from the lacI gene. *S. aureus* cells were grown in TSB, and *E. coli* strains in LB media supplemented with appropriate antibiotics as described below.

The low copy number cloning plasmid, pHSG575 (26), was kindly provided by Dr. Michael Caparon (Department of Molecular Microbiology, Washington University School of Medicine). The plasmid pBluescript KS+ was purchased from Stratagene (La Jolla, CA) and used for subcloning and sequencing purposes. The expression plasmid pQE-30 was obtained from Qiagen. All of these plasmids were propagated in DH5α cells and purified using the Qiagen Plasmid Midi-Prep Kit for further applications.

Isolation of *S. aureus* genomic DNA, preparation of probes, and Southern blotting

High molecular weight genomic DNA was isolated from 400 ml of an overnight culture of *S. aureus* strain 12598 cells by lysostaphin lysis, followed by treatment with DNase-free RNase, and subsequent purification by phenol/chloroform and chloroform extractions. After the final chloroform extraction, DNA in the aqueous layer was precipitated with ethanol and lyophilized.

A degenerate 30mer oligonucleotide probe corresponding to the amino acid sequence NNFKDDFEKN was generated by chemical synthesis. The oligonucleotide was end-labeled with T4 polynucleotide kinase and γ-^{32}P-ATP, and the radiolabeled oligonucleotide was separated from unincorprated ^{32}P by Chroma Spin-10 spin chromatography. The specific activity was approximately 5 x 10^8 cpm/μg of

oligonucleotide. The 2.6 kb Hind III/Hinc II probe was generated as described below and radiolabeled with α-^{32}P-CTP using the rediprime DNA labeling system.

Genomic and plasmid DNAs were digested to completion with restriction endonucleases. Restriction endonuclease-cleaved DNAs were separated by TAE-agarose gel electrophoresis, and Southern blotted to nitrocellulose membranes. The membranes were baked at 80°C for 2h under vacuum, and pre-hybridization, hybridization, and washing of the membranes were performed according to instructions supplied with the Rapid-hyb buffer. Washed blots were air-dried and exposed to Kodak XAR-5 films at -70°C with intensifying screens for 0.5-2 days.

Cloning and sequencing of *ebpS*

Based on the observation that the 30mer oligonucleotide probe hybridized to a 4.2 kb EcoR I-genomic DNA fragment (Fig. 1: lane A), a size selected genomic library in the 4.2 kb region was generated. Genomic DNA from *S. aureus* strain 12598 was digested with EcoR I and fractionated with 1% low melting agarose electrophoresis. The 4.2 kb region was excised from the gel and melted at 68°C for 15 min. DNA in the melted agarose was ligated in situ with pHSG575 treated with EcoR I and alkaline phosphatase according to instructions provided by FMC Products. Competent DH5α cells were transformed with the ligated material, and different dilutions were plated out on LB agar-medium plates supplemented with chloramphenicol (20 µg/ml), IPTG (0.5 nM), and X-Gal (40 µg/ml) for antibiotic and blue/white selections. White colonies were collected, propagated overnight, and the Wizard plasmid mini-prep was used to isolate plasmid DNA from cells. Purified plasmids were digested with EcoR I and screened by Southern blotting using the radiolabeled oligonucleotide probe.

The cloned 4.2 kb fragment was digested with Hind III and Hinc II, yielding a 2.6 kb fragment, which was subcloned into pBluescript KS+ and pUC19. The 2.6 kb fragment was also used as a probe in Southern analyses with *S. aureus* genomic DNA.

The insert was digested using the Exo III/mung bean nuclease system (Stratagene, La Jolla, CA) to generate two sets of nested deletions. Multiple clones covering both strands in their entirety were sequenced by the Sanger dideoxynucleotide chain termination method as modified for TAQ polymerase cycle sequencing using an ABI373A automated DNA sequencer. Sequence data were assembled and discrepancies resolved using the Wisconsin Package (Genetics Computer Group, Madison, WI). The primary sequence of *ebpS* as shown in Figure 3 has been assigned the GenBank accession number.

Expression of ebpS in *E. Coli* and CNBr cleavage of recombinant EbpS

A 2.6 kb Hind III/Hinc II fragment in pBluescript KS+(30ng) served as the template, and PCR reactions were performed with a Perkin Elmer thermocycler using standard reagents. The open reading frame of ebpS was PCR,amplified using the sense oligonucleotide, 5'-TGTGGATCCATAGAAAGGAAGGTGGCTGTG-3', and the antisense oligonucleotide, 5'GCAAAGCTTGCTGTACCAGGACCAATT-3'. The sense oligonucleotide contained a BamH I site (underlined), and A of the two ATG codons were changed to G (in bold letters) to avoid internal initiation of translation as recommended by Qiagen. The antisense oligonucleotide contained a Hind III cleavage site (underlined). The exact conditions for amplification were 90°C for 1 min. followed by 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 60 s. The PCR product was digested with BamH I and Hind III, and gel purified. This material was ligated to pQE-30 that had been digested with BamH I and Hind III and treated with calf intestinal alkaline phosphatase. Competent M15 cells were transformed with the ligation product, selected by ampicillin (100 µg/ml) and kanamycin (20 µg/ml), and antibiotic-resistant cells were screened for recombinant protein expression.

Upon obtaining several positve clones, ideal conditions for maximum expression were examined. Based on results from these studies, the following protocol was used

routinely for medium-scale purification of recombinant EbpS (rEbpS). A stock culture of the clone was grown overnight in 10 ml of LB media supplemented with ampicillin and kanamycin. On the following day, this culture was added to 100 ml of fresh LB media with antibiotics. Cells were allowed to re-grow until the OD_{600nm} value reached 0.8 (~3 h). Then expression was induced with 1 mM IPTG for 4 h at 37°C. The cells were pelleted by centrifugation (5000 x g), resuspended in 15 ml of buffer A (8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 8), and vortexed gently for 15 min. The lysed cells were centrifuged at 15,000 x g for 20 min at 4°C, and the supernatant was transferred to a tube containing 4 ml of nickel nitriloacetic acid (Ni^{++}-NTA) resin pre-equilibrated with buffer A. The mixture was incubated for 30 min at room temperature with gentle agitation. The mixture was then transferred to a disposable polypropylene column and washed consecutively with 100 ml of buffer A and 100 ml of buffer B (same as buffer A except pH=6). The tightly bound recombinant protein was eluted with 10 ml of buffer c (same as buffer A, but pH=4). The eluted material was dialyzed twice against 4L of 10 mM Tris-HCl, pH 7.5 and the concentration of the dialysate was measured by UV spectrophotometry based on the number of Tyr and Trp residues in rEbpS (1A(280nm)=2.68 mg/ml). The yield of purified rEbpS under these conditions was approximately 5 mg per 100 ml of culture.

To generate CNBr-cleaved fragments, 500 µg of rEbpS was incubated in the dark for 24 h at room temperature with 1 mg of CNBr in 200 µl of 70% formic acid. At the end of incubation, the sample was diluted with 14 ml of de-ionized H_2O and speed-vac dried. The dried material was resuspended in 10 ml of de-ionized H_2O and re-dried in 100 µg aliquots.

Generation of rabbit anti-rEbpS polyclonal antibodies

Pre-immune sera were collected, and New England White rabbits were injected with highly purified rEbpS (20µg) mixed 1:1 with complete Freunds adjuvant. Booster

injections (20 μg) mixed 1:1 with incomplete Freunds adjuvant were given at 5, 7, 10, 14, and 19 weeks. Sera were tested by Western immunoblotting using rEbpS.

IgG fractions were purified from immune and pre-immune sera by either caprylic acid precipitation (27) or protein A affinity chromatography. For generation of an antibody affinity resin, approximately 100 mg of anti-rEbpS IgG were covalently coupled to 5 ml of Affi-Gel-10 according to manufacturer's instructions. To generate anti-rEbpS Fab fragments, 50 mg of lyophilized IgGs were reacted overnight at 37°C with 2 ml of immobilized papain in 5 ml of papain digestion buffer (20 mM NaH_2PO_4, 20 mM cysteine-HCl, 10 mM EDTA, pH 6.5). Fab fragments were separated from undigested IgGs and Free Fc fragments by protein A affinity chromatography.

Binding of radiolabeled rEbpS constructs to immobilized elastin peptides

Preparation and coupling of elastin peptides to Affi-Gel-10 were as described previously (20). Both rEbpS (20 μg) and CNBr-cleaved rEbpS (80 μg) were iodinated with 300 μCi of $Na^{125}I$ by the IODOGEN method. The specific activities were approximately 2.3×10^4 and 1.2×10^4 cpm/ng protein for rSEBP and CNBr-cleaved rEbpS fragments, respectively. Radiolabeled rEbpS (45 ng) in 1.5 ml of binding buffer (50 mM Tris, 500 mM NaCl, 2 nM $CaCl_2$,0.1 mg/ml BSA, pH 7.5) was incubated with 1 ml of the elastin peptide affinity resin for 2 h at room temperature in the absence or presence of 2 mg unlabeled elastin peptides. The mixture was transferred to disposable polypropylene columns and washed with binding buffer by gravity flow until radioactivity of the flow through reached background. Bound rEbpS was eluted with 3 ml of 1% SDS buffer, spin concentrated, and analyzed by 10% SDS-PAGE and autoradiography. Binding of radiolabeled CNBr-cleaved rEbpS to immobilized elastin was assessed similarly, except 80 ng of the starting material was used and bound material was visualized by 12% SDS-PAGE and autoradiography.

Detection of the native 25 kDa cell surface labeled EbpS with anti-rEbpS antibodies

Surface labeled extracts from *S. aureus* cells were prepared by lysotaphin digestion as described previously (20). Approximately 10^7 cpm of surface labeled extract was first absorbed with 3 ml of pig IgG-Affi-Gel 10 resin for 2 h at room temperature. The unbound supernatant was collected by centrifugation and incubated with 1 ml of the anti-rEbpS IgG affinity resin in the absence or presence of 2 mg unlabeled rEbpS for 2 h at room temperature in 2 ml of binding buffer. The mixtures were transferred to disposable columns and washed with binding buffer until flow through reached background radioactive levels. Bound cell surface-labeled molecules were eluted from the column by 3 ml of 1% SDS buffer, spin-concentrated, and analyzed by 15% SDS-PAGE and autoradiography.

Other procedures

Purification and radiolabeling of full length recombinant human elastin, and cellular elastin binding assays were performed as described previously (20). Automated amino acid sequence and composition analyses were carried out in our laboratory by the Applied Biosystems 473A protein sequencer and Beckman System 6300 High Performance Analyzer, respectively. Electron spray mass spectrometry was performed by the Protein Chemistry Laboratory at Washington University School of Medicine, St. Louis, MO.

Results

Cloning of ebpS

The N-terminal sequence of native EbpS expressed on the cell surface of *S. aureus* was determined previously to be ANNFKDDFEKNRQ (20). A degenerate oligonucleotide corresponding to residues 2-11 of the determined N-terminal sequence was generated and used as a probe. Southern blot analysis was first

performed with *S. aureus* strain 12598 genomic DNA digested with restriction endonucleases to identify the hybridizing genomic fragment. As shown in Figure 1, the oligonucleotide probe hybridized to a 4.2 kb EcoR I fragment (lane A). On the basis of this observation, a size-selected genomic plasmid library in the 4.2 kb region was constructed from EcoR I-digested *S. aureus* genomic DNA and screened with the oligonucleotide probe by Southern blotting. Of 120 colonies screened, two positive clones with identical restriction enzyme digestion patterns were isolated. One of these clones, pEBPS-1, was used for further analysis.

To determine whether the correct 4.2 kb fragment was cloned, the radiolabeled oligonucleotide was hybridized to the pEBPS-1 insert, and the cloned insert itself was used as a probe for Southern analyses with EcoR I-and EcoR I/Hind III/Hind II-digested genomic DNA. The oligonucleotide probe hybridized to the 4.2 kb pEBPS-1 insert (Fig. 1: lane B) and the insert recognized a 4.2 kb EcoR I genomic fragment (Fig. 1: lane C). With EcoR I/Hind III/Hinc II-digested genomic DNA, the radiolabeled pEBPS-1 insert hybridized to a 2.6 kb fragment (Fig. 1: lane D). The oligonucleotide and cloned insert probes consistently detected single fragments with identical size in Southern analyses using genomic DNA digested with various restriction endonucleases, indicating that ebpS is present as a single copy gene.

Analysis of the primary sequence of *ebpS*

pEBPS-1 was digested with Hind III and Hinc II to yield a 2.6 kb fragment. This fragment was subcloned into pBluescript II KS+ to generate pKS-2.6 and sequenced to locate an open reading frame containing the N-terminal sequence of cell surface EbpS. A 606 bp open reading frame which starts with an ATG codon was identified about 0.9 kb 3' of the Hind III site. The physical map of pKS-2.6 and the primary sequence of the open reading frame with up- and downstream sequences are shown in figures 2 and 3, respectively. Putative -10 and -35 hexamers were identified at positions -31 and -54, with a spacing of 17 bp. A third AT-rich promoter sequence

has been proposed recently to exist in a region about 20 bp upstream of the -35 hexamer in *E. coli* (28), and this region for ebpS was 75% AT. A potential ribosome binding sequence, which complemented perfectly with the extreme 3' region of Bacillus subtilis 16S RNA (UCUUUCCUCC) (29), was found at position -7. Overall, ebpS was 64% AT and 36% CG. Although two ATG codons were found in the correct reading frame of ebpS, we have designated the second ATG as the initiation codon based on the location of the putative ribosome binding site. The N-terminal sequence of cell surface EbpS determined from peptide sequencing was found to start at the second residue of the predicted sequence, suggesting that the initial Met residue is cleaved. The deduced sequence matched perfectly with the determined sequence of cell surface EbpS except for the first amino acid (Ala in native, Ser in deduced). Since Ser residues are often misread because of its small peak in the peptide sequencing chromatogram, we reexamined the original sequencing chromatogram of cell surface EbpS and have identified clear Ser and Ser' peaks indicating that the residue in concern is a Ser and not Ala.

The mature protein has a predicted molecular mass of 23,344.7 daltons and an acidic pI of 4.9. Accordingly, the protein has a preponderance of acidic amino acids Asp (10.9%) and Glu (11.9%), but, is devoid of Cys residues. Garnier analysis predicts a secondary structure that is 58.4% α helical and 23.8% coiled coil. The BLAST network service of the NIH on the internet was used to search for sequence homologies. The May 1, 1995 releases of the Brookhaven Protein Data Bank, GenBank, EMBL Data Library, SWISS-PROT protein sequence database, and the trasnlated coding sequence of GenBank were used for comparison. No significant homologies were found between reported sequences in these databases and the primary sequence of ebpS.

Expression of ebpS in *E. coli*

We studies whether the cloned gene encodes an elastin binding protein by expressing ebpS in *E. coli*. The PCR-amplified ebpS open reading frame was expressed in *E. coli* as a fusion protein containing six His residues tagged to the N-terminus of the protein. Recombinant EbpS (rEbpS) was purified from *E. coli* extracts by Ni^{++}-NTA chromatography based on the high affinity binding interaction between Ni^{++} and His residues. As can be seen in figure 4, the three positive clones expressed large amounts of homogeneous rEbpS. However, purified rEbpS from all three clones migrated as a 45 kDa protein when fractionated by SDS-PAGE, which was a significant deviation from its predicted molecular mass of 26 kDa. Based on the size difference and the fact that rEbpS was fractionated by reducing SDS-PAGE, it was unlikely that the observed abnormal migration was caused by dimerization of rEbpS.

To examine the fidelity of rEbpS, the N-terminal sequence of full length rEbpS, as well as internal sequences from a degradation product and two fragments generated by CNBr cleavage were determined by protein microsequencing. Altogether, an unambiguous sequence was obtained for 58 residues, and they matched perfectly with the predicted sequence Fig. 2 underlined sequences). Furthermore, amino acid and mass spectrometry analyses indicated that the composition of rEbpS and actual molecular mass of rEbpS, respectively, are in agreement with the predicted data. These results indicate that the correct protein has been expressed, and that overestimation of the molecular mass is due to aberrant migration in SDS-PAGE.

Elastin binding activities of rEbpS constructs

To investigate whether rEbpS interacts specifically with elastin, elastin peptide affinity chromatography was performed with radiolabeled rEbpS. Iodinated rEbpS was incubated with the elastin peptide affinity resin for 2 h at room temperature in the absence or presence of excess unlabeled elastin peptides. The mixture was then washed extensively with buffer until radioactivity of the flow through reached background. The bound material was eluted with 1% SDS buffer and analyzed by

SDS-PAGE and autoradiography. The starting material for this experiment was stored for one week at 4°C after purification with Ni^{++}-NTA chromatography. As can be seen in Figure 5, the starting material for this experiment was partially degraded (lane B). Because the sequence of the 40 kDa major degradation product starts after an Arg residue (Fig. 2, starts at residue #60), a trypsin-like enzyme is the likely cause of degradation. This partially degraded material lacking the first 59 amino acids did not associate with the affinity resin. In contrast, the full length form of labeled rEbpS interacted efficiently with the elastin peptide affinity resin in the absence (Fig. 5: lane C), but not in the presence, of excess unlabeled elastin peptides (lane D). These results suggest that the 59 amino acid region plays a critical role in elastin recognition.

To further study the hypothesis that the N-terminal region of EbpS contains the ligand binding site, elastin binding properties of CNBr-cleaved rEbpS fragments were examined since the presence of a single internal Met residue is predicted from DNA sequence data. In agreement with agreement with the predicted sequence, two dominant bands were detected in the radiolabeled starting material of CNBr-cleaved rEbpS. Peptide microsequencing was employed to verify correct cleavage, and to identify the N- and C-terminal fragments (Fig. 3: underlined). When elastin binding activities of these fragments were assayed with elastin peptide affinity chromatography, only the N-terminal fragment bound tightly to the elastin peptide affinity resin. These findings are consistent with the elastin binding site contained in the first 59 amino acids of EbpS.

Effects of rEbpS on *S. aureus* binding to elastin

If EbpS is the cell surface molecule responsible for elastin binding at the cellular level, then an active form of soluble EbpS should interfere with *S. aureus* binding to elastin. We tested this hypothesis by examining the effects of rEbpS on *S. aureus* binding to elastin. Elastin labeled with ^{125}I was incubated with *S. aureus* cells in the absence or presence of varying concentrations of unlabeled rEbpS for 1 h at room

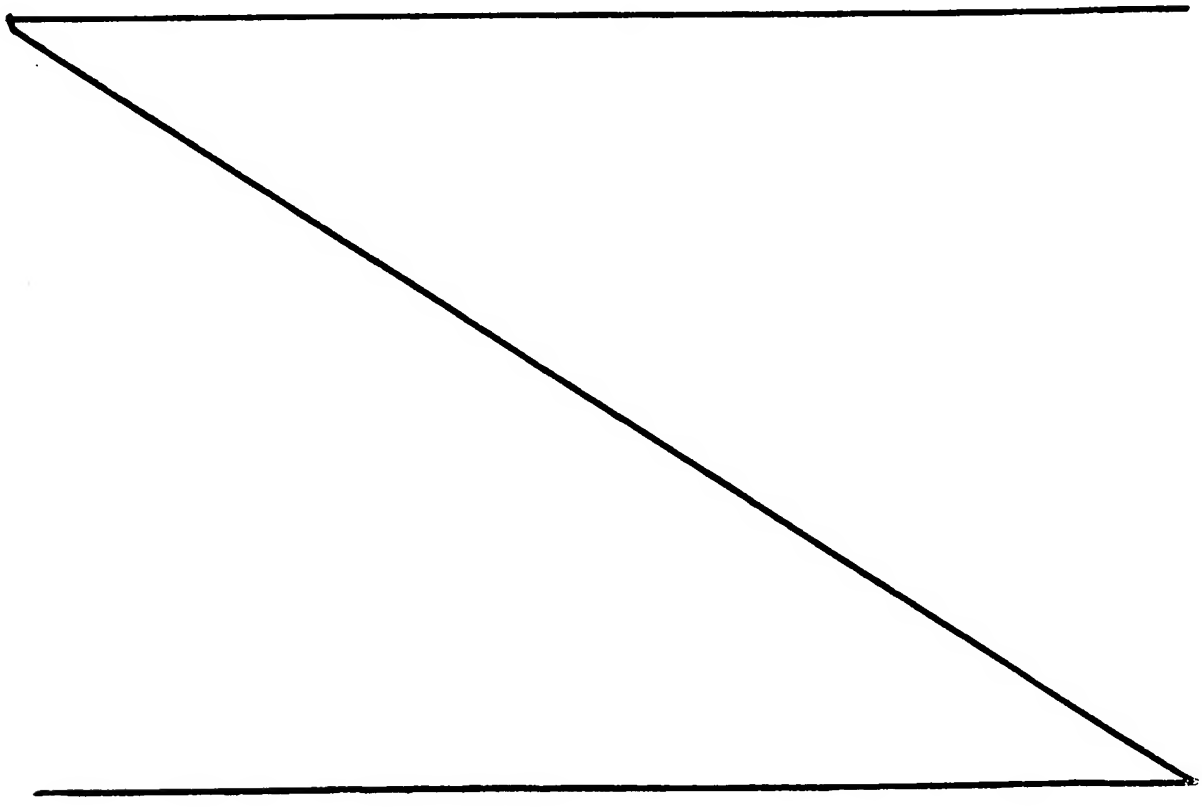
temperature in 200 µl of TSB. After washing three times with TSB, radioactivity associated with the cellular pellet was measured with a gamma counter. As can be seen in figure 6, rEbpS inhibited binding of labeled elastin in a concentration dependent manner. Furthermore, *S. aureus* binding to radiolabeled elastin was abrogated at the highest concentration of rEbpS tested (19 µM). The control polyhistidine fusion protein mouse dihydrofolate reductase (DHFR) did not affect binding at 26 µM. These results demonstrate that rEbpS inhibition of cellular elastin binding is specific and that the polyhistidine domain of rEbpS is not affecting binding.

Expression of EbpS on the cell surface of *S. aureus*

The abilities of rEbpS to interact directly with elastin and to inhibit cellular elastin binding strongly suggest that EbpS is the cell surface protein mediating *S. aureus* binding to elastin. To provide further evidence that EbpS is a cell surface protein, affinity chromatography was performed with surface labeled *S. aureus* extracts and immobilized anti-rEbpS IgGs. *S. aureus* cells were surface labeled by the IODOGEN method and extracts were prepared by lysostaphin digestion. Approximately 10^7 cpm of this material was pre-absorbed to a pig IgG affinity resin to remove surface-labeled protein A, and the flow through was incubated with the anti-rEbpS IgG affinity resin for 2 h at 25°C. After washing extensively with binding buffer, bound cell surface molecules were eluted with 1% SDS buffer and anlayzed by SDS-PAGE and autoradiography. As shown in Figure 7, pre-absorption with the pig IgG resin removed surface labeled protein A from the starting material (compare 50 kDa band in lanes A and B). Of the remaining numerous surface labeled proteins, a 35 and 25 kDa protein associated with the anti-rEbpS IgG affinity resin (lane C). To determine the specificity of binding, the same experiment was performed in the presence of excess unlabeled rEbpS. As can be seen in lane D, binding of the surface 25 kDa protein, but not the 35 kDa protein, to immobilized anti-rEbpS IgG was inhibited by unlabeled rEbpS. Densitometric scanning of the bands revealed that the band intensity for the 25 and 35 kDa protein, but not the 35 kDa protein, to immobilized anti-rEbpS IgG

was inhibited by unlabeled rEbpS. Densitometric scanning of the bands revealed that the band intensity for the 25 and 35 kDa proteins decreased by 64 and 7%, respectively, in the presence of excess unlabeled rEbpS. These results indicate that the 25 kDa protein is cell surface EbpS and that the 35 kDa protein is interacting with the agarose affinity support of the elastin peptide affinity resin.

Because the anti-rEbpS antibody is polyclonal, we reasoned that some of the antibodies may recognize a region in cell surface EbpS that is critical for elastin binding. To test this idea, effects of anti-rEbpS IgG Fab fragments on *S. aureus* binding to radiolabeled elastin binding were examined. *S. aureus* cells were incubated with labeled elastin in the absence or presence of immune or pre-immune Fab fragments. As shown in Figure 8, Fab fragments from immune IgGs inhibited binding of *S. aureus* to radiolabeled elastin in a concentration-dependent manner. In contrast, Fab fragments from pre-immune antibodies did not affect binding at the two concentrations tested.



DISCUSSION

Cell surface components of pathogenic bacteria play important roles in surviving the hostile environment of the host. For gram positive bacteria these surface molecules are used in pathogenic processes such as evading host immune responses (30), digesting host carbohydrates to expose host attachment sites (31, 32), capturing host enzymes to digest host tissues (33), and binding host tissue determinants to establish a firm basis for colonization (34). Cell surface adhesins and MSCRAMMS interact with host ECM components, and participate in the colonization of and extravasation through tissues and organs. Previously it was demonstrated that *S. aureus* binds specifically to elastin. Results from binding assays at the cellular level suggested the existence of a single type of cell surface elastin binding protein that mediates the *S. aureus*-elastin binding interaction. Accordingly, cell surface EbpS with similar binding properties to *S. aureus* binding to elastin was isolated. On the basis of these findings, EpbS has been proposed to be the elastin MSCRAMM. The focus of this study was to examine this hypothesis by studying the molecular structure and function of EbpS.

The present results demonstrate that the 606 bp ebpS exists as a single copy gene in the *S. aureus* genome, and that the primary sequence of *ebpS* is novel. Mature EbpS has a predicted molecular mass of 23 kDa and is highly charged. Furthermore, soluble EbpS expressed in *E. coli* interacts directly with elastin. Sequence, composition, and size fidelities of rEbpS were analyzed by peptide microsequencing, amino acid analysis, and mass spectrometry and determined to be correct. The recombinant protein with an actual size of 26 kDa, however, migrates aberrantly as a 45 kDa protein in SDS-PAGE. Abnormal migration in SDS-PAGE has been observed frequently for proteins expressed as a polyhistidine fusion protein and also for numerous gram positive cell surface proteins (35-39). In some cases, the aberrant migration of gram positive surface proteins has been attributed to the presence of multiple repetitive domains (35,36) and high Pro content (37,38) of these proteins.

The abnormal migration of rEbpS cannot be due to these factors because rEbpS lacks multiple repetitive domains and does not have a high Pro content (3.6% of total). Since acidic amino acids are uniformly abundant in EbpS (pI=4.9), a likely explanation may be that the surface negative charge of rEbpS is hindering the uniform attachment of anionic SDS and causing the observed abnormal migration in SDS-PAGE.

Several independent criteria indicate that EbpS is the surface protein mediating cellular elastin binding. First, rEbpS binds specifically to immobilized elastin and inhibits binding of *S. aureus* cells to elastin in a dose dependent manner. These results establish that EbpS is an elastin binding protein that is functionally active in a soluble form. Second, an antibody raised against rEbpS recognizes a 25 kDa protein expressed on the cell surface of *S. aureus* cells. In addition to the size similarity and antibody reactivity, further evidence that this 25 kDa protein is cell surface EbpS is provided by the experiment showing that binding of the 25 kDa protein to immobilized anti-rEbpS IgG is inhibited in the presence of excess unlabeled rEbpS. Finally, Fab fragments prepared from the anti-rEbpS antibody, but not from its pre-immune control, inhibit binding of *S. aureus* to elastin. This result suggests that the topology of surface EbpS is such that the elastin binding site is accessible to interact with ligands (i.e. elastin and the anti-rEbpS Fab fragment) and not embedded in the cell wall or membrane domains. The composite data demonstrate that EbpS is the cell surface protein responsible for binding *S. aureus* to elastin.

Although the present results indicate that EbpS is expressed on the cell surface, little is known regarding the mechanism involved in surfaced expression of the protein. Several surface proteins of gram positive bacteria have been found to share common motifs important in sorting, transporting, and anchoring these proteins to the cell surface (40). The common features consist of a cleaved signal peptide, which is followed by the ligand binding extracellular N-terminal domain, a Pro-rich region thought to span the cell wall, a conserved LPXTGX hexapeptide sequence, a

hydrophobic membrane spanning domain, and a charged C-terminal tail. A recent study by Schneewind et al. (41) have shown that at least for protein A of *S. aureus*, the common structure is cleaved after the Thr residue of the hexapeptide sequence and the protein is anchored to the cell wall via amide linkage of the carboxyl group of Thr and free amino group of the pentaglycine peptide moiety of the staphylococcal peptidoglycan. Apart from the putative localization of the elastin binding site to the extracellular N-terminal domain and identification of a charged C-terminal tail, our results show that EbpS does not contain other common motifs. This observation, however, is not unique for EbpS in that several other gram positive surface proteins have been found to deviate from this conserved structure. Although we have not identified any sequence or structural similarities, EbpS and other proteins not conforming to the common structure are generally smaller than the majority of proteins expressing the shared motifs. The list includes streptococcal proteins such as the fibronectin/fibrinogen binding protein (54 kDa) (42), albumin binding protein (36 kDa)(38), and the plasmin receptor (36 kDa) (43). Furthermore, similar to EbpS, the initial Met residue of the streptococcal plasmin receptor is cleaved in the mature protein (43). It is not known whether these correlations have a role in an alternative mechanism for surface expression.

The present and previous findings suggest the existence of a functionally active 40 kDa intracellular precursor form of EbpS that requires processing at the C-terminus prior to surface expression. This notion is based on the following observations: i) there exists an intracellular 40 kDa elastin binding protein that is never detected during cell surface labeling experiments, ii) the 25 kDa EbpS and the 40 kDa elastin binding protein have an identical N-terminal sequence, and iii) a single gene exists for EbpS. Because the size of the *ebpS* open reading frame is not sufficient to encode a 40 kDa protein, at first we disregarded this hypothesis. However, our studies with rEbpS demonstrated that although the actual size of the recombinant protein is 26 kDa, it migrates aberrantly as a 45 kDa protein in SDS-PAGE. This finding suggests that full length native EbpS, with a predicted size of 23 kDa, may be migrating in

SDS-PAGE as the 40 kDa intracellular precursor, and that the 25 kDa surface form of EbpS is actually a smaller form of the molecule processed at the C-terminus. Although EbpS lacks an N-terminal signal peptide and other known sorting and anchoring signals, this proposed intracellular processing event may explain some questions regarding how EbpS is targeted to the cell surface. In fact, C-terminal signal peptides have been identified in several bacterial proteins (44) and alternative means of anchoring proteins to the cells surface have been reported in gram positive bacteria (45). The C-terminus of EbpS may be processed intracellularly, although where and how this cleavage occurs, and if this processing event is one of the signals required for surface expression of EbpS, is yet to be ascertained.

It is now apparent that interactions between pathogenic bacteria and host ECM components are playing important roles in disease pathogenesis. However, molecular structure:function analyses for most ECM adhesins have not been performed despite the obvious potential of developing effective prophylactic and therapeutic agents based on information derived from these studies. In cases where investigations have been performed, the primary ligand binding site has been found to be contained in the C-terminal end of the extracellular N-terminal domain (36,46,47). The present results showing lack of elastin binding activity by the C-terminal fragment of CNBr-cleaved rEbpS and inhibition of cellular elastin binding by the anti-rEbpS Fab fragments suggest that the elastin binding site in EbpS is also contained in the extracellular N-terminal domain. Further mapping of the putative elastin binding site within this region to the first 59 amino acids of EbpS is based on the finding that a degradation product of rEbpS lacking these residues failed to interact with elastin. Similar to other domains in EbpS, the putative elastin binding region in EbpS lacks homology with sequences reported to various databases. A truncated, recombinant construct of EbpS containing this 59 amino acid domain inhibits binding of *S. aureus* to elastin. These observations indicate that the 59 amino acid region contains the elastin binding site of EbpS.

EXAMPLE 2

Cell-extracellular matrix (ECM) interactions are necessary events in various biological processes including embryonic development, inflammation, tumor cell metastasis, homeostasis, and microbial infections [Bernfield, *et al., Annu. Rev. Cell Biol.*, **8**:365-393 (1992); Liotta, *et al., Annu. Rev. Biochem.*, **55**:1037-1057 (1986); Patti, *et al., Annu. Rev. Microbiol.*, **48**:585-617 (1994)]. Molecular interactions between ECM components and corresponding cell surface receptors instruct cells to differentiate, migrate, adhere, or proliferate in directing these biological processes. Although there are exceptions, mammalian cells typically use the integrins, an α/β heterodimeric receptor complex, to interact with the ECM [Hynes, *Cell*, **69**:11-25 (1992); Albelda, *et al., FASEB J.*, **4**:2868-2880 (1990)].

Bacterial pathogens also interact with the host matrix through specific cell surface ECM binding molecules categorized collectively as adhesins or MSCRAMMS [Patti, *et al.*, 1994, *supra*; Höök, *et al., Cell Differ. Dev.*, **23**:433-438 (1990)]. The gram positive bacterial pathogen *Staphylococcus aureus* (*S. aureus*) has been found to interact with many ECM macromolecules such as collagen [Holderbaum, *et al., Infect. Immun.*, **54**:359-364 (1986); Speziale, *et al., J. Bacteriol.*, **167**:77-81 (1986)], fibronectin [Kuusela, *Nature*, **276**:718-720 (1978)], laminin [Lopes, *et al., Science*, **229**:275-277 (1985)], proteoglycans [Liang, *et al., Infect. Immun.*, **60**:899-906 (1992)], fibrinogen [McDevitt, *et al., Mol. Microbiol.*, **11**:237-248 (1994)], and elastin [Park, *et al., J. Biol. Chem.*, **266**:23399-23406 (1991)]. While *S. aureus* adhesins bind host ECM, these interactions have different biological purposes and binding profiles from mammalian ECM receptors. In general, Staphylococcal ECM adhesins are used for pathological purposes such as in colonization of host tissues. For example, collagen [Patti, *et al., Infect. Immun.*, **62**:152-161 (1994)] and fibronectin [Baddour, *Infect. Immun.*, **62**:2143-2148 (1994)] adhesin mutants show a reduced capacity to cause disease in *in vivo* models, but are otherwise phenotypically normal.

At the molecular level, all characterized staphylococcal adhesins function as monomers, and endogenous bacterial ligands have not been identified. Available evidence suggests that ligand binding sites in staphylococcal ECM adhesins are contained within small regions of the extracellular domain. The ligand binding site in the staphylococcal fibronectin binding protein, for example, has been mapped to a repetitive 38 amino acid motif, and corresponding synthetic peptides have been found to possess direct binding activity and to inhibit bacterial binding to fibronectin [Signäs, *et al., Proc. Natl. Acad. Sci. USA,* **86**:699-703 (1989); Raja, *et al., Infect. Immun.,* **58**:2593-2598 (1990)]. Similarly, a synthetic 25 amino acid peptide corresponding to the region between Asp^{209} and Tyr^{233} of the collagen adhesin has been shown to inhibit binding of type II collagen to *S. aureus* [Patti, *et al., J. Biol. Chem.,* **270**:12005-12011 (1996)], suggesting that this short peptide sequence alone can mediate staphylococcal binding to collagen.

Specific binding between *S. aureus* and elastin is mediated by a 25 kDa elastin binding protein (EbpS) on the surface of *S. aureus* [Park, *et al.,* 1991, *supra*]. Elastin binding activity is localized to the extracellular, amino terminal end of EbpS within the first 59 amino acids [Park, *et al.,* 1991, *supra*; Park, *et al., J. Biol. Chem.,* **271**:15803-15809 (1996)]. To better define the amino acids in EbpS responsible for the elastin binding activity, overlapping synthetic peptides and truncated, recombinant EbpS constructs were used in elastin binding assays. Our results demonstrate that the critical elastin recognition sequence within the N-terminal domain resides between Gln^{14} and Glu^{34} SEQ ID NO: 13. Sequence comparison indicates that the minimal sequence shared by all active EbpS constructs is the hexamer Thr-Asn-Ser-His-Gln-Asp spanning residues 18-23 (SEQ ID NO: 16).

Materials and Methods

Materials

Restriction enzymes, calf intestinal alkaline phosphatase, T4 DNA ligase, isopropyl-β-thiogalactoside, 5-bromo-4-chloro-3-indolyl-β-D-galactoside, and Hind III-digested λ DNA markers were purchased from Promega (Madison, WI). Luria-Bertani medium and Luria-Bertani agar-medium capsules were from BIO 101 (La Jolla, CA). Tryptic soy broth (TSB) was obtained from Remel (Lenexa, KS). $Na^{125}I$ was from ICN (Costa Mesa, CA). Papain and protein A immobilized to cross-linked agarose, Immunopure Sulfo-NHS-Biotinylation kit, and IODOGEN were purchased from Pierce (Rockford, IL). QIAexpress vector kit type IV and the midi-prep plasmid purification kit were obtained from Qiagen (Chatsworth, CA). Nitrocellulose membrane and blotting paper were from Schleicher & Schuell (Keene, NH). Affi-Gel-10 affinity support was from Bio-Rad (Melville, NY). All other materials were purchased from Sigma Chemical (St. Louis, MO).

Synthetic peptides

Synthetic peptides containing the deduced primary sequence of EbpS were prepared by conventional solid phase synthesis on an Applied Biosystems model 431A synthesizer using FastMoc chemistry. Peptides were purified by reverse phase high performance liquid chromatography (Beckman C18, 0-80% linear water-acetonitrile gradient containing 0.05% trifluoroacetic acid). Purity of the peptides were confirmed by either amino terminal sequencing or electron spray mass spectrometry. All synthetic peptides were soluble in the assay buffer at the concentrations tested.

Expression of recombinant EbpS proteins

Expression of full length recombinant EbpS (rEbpS) as a fusion protein with a polyhistidine tag at the N-terminus was as previously described [Park, *et al.*, 1996, *supra*]. Essentially the same protocol was followed to make truncated recombinant EbpS-1 and 2 (trEbpS-1 and trEbpS-2), except that different PCR primers were used. For the generation of trEbpS-1 containing EbpS residues 1-78, a 2.6 kb *Hind* III/*Hinc*

II fragment in pBluescript KS+ which contains full length *ebpS* was PCR-amplified using the oligonucleotide 5'-TGTGGATCCATAGAAAGGAAGGTGGCTGTG-3' as the forward primer and the oligonucleotide 5'-CATTGAGCTCAGATGTTTGTGATTC-3' as the reverse primer. To make trEbpS-2 corresponding to amino acid residues 1-34 of EbpS, the same template and forward primer were used, whereas the oligonucleotide 5'-GTTCGAGCTCTGATTGGTCTTTTTC-3' served as the reverse primer. The forward primer contained a *Bam*H I site (underlined), and A of the two ATG codons was changed to G (in bold letters) to avoid internal initiation of translation as recommended by Qiagen. The reverse primers contained a Sac I cleavage site (underlined). PCR amplification was performed with a Perkin Elmer thermocycler using standard reagents. Conditions for PCR amplification, and subsequent restriction enzyme digestion, ligation, transformation, expression, and purification of recombinant proteins were as previously described [Park, *et al.*, 1996, *supra*].

Direct binding of trEbpS proteins to elastin

trEbpS-1 and trEbpS-2 were biotinylated using a commercially available kit (Pierce). Recombinant proteins (1 mg) were incubated with sulfo-NHS-biotin reagent (2 mg) in 1 ml of PBS for 2 h at 4°C. Biotinylated proteins were then separated from free biotinylating reagent by PD-10 gel filtration chromatography.

Three micrograms of recombinant human tropoelastin, bovine serum albumin (BSA), and ovalbumin were fractionated by 10% SDS-PAGE and transferred to nitrocellulose membranes by Western blotting. Transferred blots were blocked overnight at 4°C with blocking buffer which contained 0.5% (w/v) BSA and 0.05% (v/v) Tween-20 in Tris-buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5). The blots were washed twice with blocking buffer and incubated for 2 hours at room temperature with either 5 μM biotinylated trEbpS-1 or trEbpS-2 in the absence or presence of 3 mg/ml elastin peptides in blocking buffer. After washing twice with blocking buffer,

the blots were incubated with a 1:1000 dilution of avidin conjugated to horseradish peroxidase. Membranes were developed by 4-chloro-naphthol.

EbpS polyclonal antibodies

Immunization protocols and characterization of the anti-EbpS polyclonal antibodies have been described previously [Park, *et al.*, 1996, *supra*]. To subtract the population of antibodies recognizing EbpS residues 1-34, anti-EbpS IgGs (25 mg) purified by caprylic acid precipitation [McKinney, *et al., J. Immunol. Methods,* **96**:271-278 (1987)] were incubated overnight at 4°C with trEbpS-2 (8 mg) coupled to 3 ml of Affi-Gel-10. On the following day, the mixture was transferred to a disposable polypropylene column, and unbound IgGs were collected by gravity flow. The efficiency of the absorption was tested by Western immunoblotting against the full length and two truncated constructs of recombinant EbpS (Fig. 2c). Fab fragments from both the original and trEbpS-2-absorbed IgG fractions were prepared by digestion with immobilized papain as described previously [Park, *et al.*, 1996, *supra*].

Other procedures

Purification and radiolabeling of full length recombinant human soluble tropoelastin, generation of elastin peptides, cellular elastin binding assays, SDS-PAGE, and Western blotting were performed as described previously [Park, *et al.*, 1996, *supra*]. Automated amino acid sequencing was performed using an Applied Biosystems 473A protein sequencer. Electron spray mass spectrometry was performed by the Protein Chemistry Laboratory at Washington University School of Medicine (St. Louis, MO). The PROTEAN program (DNAStar, Madison, WI) was used to predict the secondary structure of EbpS constructs.

Results

Truncated recombinant EbpS constructs bind to elastin and inhibit *S. aureus* binding to elastin

Results from studies of elastin binding properties of various EbpS fragments [Park, *et al.*, 1996, *supra*] and recombinant constructs are summarized in Figure 9. A cyanogen bromide fragment containing the first 125 amino acids of EbpS showed binding activity, whereas an EbpS degradation product lacking the first 59 amino acids, a cyanogen bromide fragment containing the C-terminal one-third of the protein and a synthetic peptide corresponding to residues 1-13 did not interact with elastin. These results suggested that the elastin binding site in EbpS is contained in amino acid residues 14-59 SEQ ID NO:15 (shaded area in Figure 1).

To identify the amino acids critical for binding, two truncated recombinant constructs of EbpS (trEbpS-1 having a nucleic sequence of SEQ ID NO:9, and trEbpS-2 having a nucleic acid sequence of SEQ ID NO:11) were generated and the corresponding polypeptides were tested both for their ability to directly bind elastin and to inhibit binding of *S. aureus* to elastin. trEbpS-1, with a predicted molecular mass of 12.8 kDa, contains residues 1-78 of EbpS (SEQ ID NO:10), whereas trEbpS-2 spans residues 1-34 (SEQ ID NO:12) and has a predicted mass of 7.5 kDa. Characterization of the truncated constructs by mass spectrometry, peptide microsequencing, and immunoblotting with the anti-rEbpS antibody (Fig. 10B, lanes C and D) confirmed that correct truncated proteins have been expressed. Both proteins migrated at higher than predicted molecular weights when fractionated by SDS-PAGE (Fig. 10). This behavior has also been observed with full length recombinant EbpS (rEbpS) SEQ ID NO:2. Aberrant migration in SDS-PAGE appears to be a common characteristic of gram positive cell surface proteins [McDevitt, *et al.*, 1994, *supra*; Signäs, *et al.*, 1989, *supra*; Murphy, *et al.*, *Biochem. J.*, **277**:277-279 (1991); Sela, *et al.*, *Mol. Microbiol.*, **10**:1049-1055 (1993); Sjöbring, *Infect. Immun.*, **60**:3601-3608 (1992); Talay, *et al.*, *Mol. Microbiol.*, **13**:531-539 (1994)].

To examine whether trEbpS-1 and trEbpS-2 bind directly to elastin, the truncated proteins were biotinylated and reacted with tropoelastin that was transferred to nitrocellulose membranes. As shown in Figure 11, both truncated constructs bound to the 67 kDa tropoelastin in the absence (lanes A and C), but not in the presence (lanes B and D), of competing soluble elastin peptides. The biotinylated proteins did not bind to either ovalbumin or BSA under similar conditions, demonstrating that the binding interaction between the two polypeptides, *i.e.*, trEbpS-1 and trEbpS-2, and tropoelastin is specific.

Effects of trEbpS constructs on elastin binding at the cellular level were tested by incubating *S. aureus* cells with radiolabeled elastin in the absence or presence of increasing amounts of either soluble full length (rEbpS) or truncated forms of the receptor. All three polypeptides inhibited binding of *S. aureus* cells to elastin in a concentration dependent manner (Fig. 12). rEbpS and trEbpS-1 completely inhibited elastin binding at the highest concentration tested. trEbpS-2 was somewhat less effective as an inhibitor, with about 20% residual elastin binding activity at the highest inhibitor concentration.

Pre-absorption of the anti-rEbpS antibody with trEbpS-2 neutralizes its inhibitory effect

Fab fragments of a polyclonal antibody raised against rEbpS inhibit binding of *S. aureus* to elastin [Park, *et al.*, 1996, *supra*], suggesting that a population of antibodies in the immune serum recognize a region in EbpS critical for elastin binding. To test this possibility, anti-rEbpS IgGs were absorbed to the trEbpS-2 construct coupled to Affi-Gel 10, and unbound IgGs were collected. Immunoblotting revealed that the trEbpS-2-absorbed immunoglobulins retained the ability to interact with both trEbpS and the full length rEbpS-1 (Fig. 10C, lanes B and C), although with reduced activity. The immunoglobulin fraction that was not absorbed to the trEbpS-2 construct did not react with trEbpS-2 on Western blot (Fig. 10C, lane D).

Fab fragments from both the nonabsorbed and trEbpS-2-absorbed immunoglobulins were generated by papain digestion and tested for their effects on staphylococcal elastin binding. Consistent with previous findings, Fab fragments from the original anti-rEbpS IgGs abrogated the binding of *S. aureus* to elastin (Fig. 13). In contrast, serum preabsorbed with trEbpS-2 inhibited the binding of *S. aureus* to elastin by only 30% at the highest concentration tested (300 μg/ml).

Contiguous synthetic EbpS peptides inhibit *S. aureus* binding to elastin

The findings described above suggest that the elastin recognition domain in EbpS is contained within residues 14-34. To more precisely define the elastin binding site, overlapping synthetic peptides within this region were generated (Figure 14) and tested for their ability to inhibit binding to staphylococcal elastin. Repetitive sequences in EbpS were searched to find a candidate elastin binding site, since several staphylococcal and streptococcal ECM adhesins have been shown to use repetitive domains for ligand recognition [Signäs, *et al.*, 1989, *supra*; Sela, *et al.*, 1993, *supra*]. Although no identical repetitive sequences were identified, there are two related sequences, ^{21}HQDHTEDVE^{29}, SEQ ID NO:20 and ^{37}HQDTIENTE^{45}, SEQ ID NO:23, in the amino-terminal end of the molecule. The sequence ^{21}HQDHTEDVE^{29} is within the putative amino terminal elastin binding site and is contained in all active EbpS constructs. The second sequence, ^{37}HQDTIENTE^{45}, is present only in full length EbpS, and in trEbpS-1, which does not actively bind tropoelastin.

To determine whether the HQDHTEDVE sequence might participate in elastin binding, we generated two synthetic 17 amino acid peptides, P1 (SEQ ID NO:18) and P2 (SEQ ID NO:19), corresponding to residues 18-34 (Fig. 14). The P1 peptide was made according to the deduced sequence of EbpS. In the P2 peptide, Asp^{23}, Glu^{26}, and Glu^{29} were substituted with Asn, Pro, and Gln, respectively. The charged amino acids were targeted for substitution because staphylococcal elastin binding has been shown to involve electrostatic interactions [Park, *Cell Biology*, 1-161 (1993)]. As

shown in figure 15, the P1 peptide inhibited the binding of *S. aureus* to elastin in a concentration dependent manner. Elastin binding was abrogated at the highest concentration of P1, whereas minimal inhibition (~15%) was observed with the P2 peptide.

To define the elastin binding activity more completely, three overlapping 10-mers spanning amino acid residues 14-36 were generated (Fig. 14) and tested for their ability to inhibit staphylococcal binding to elastin. Peptide P4 (SEQ ID NO:21 residues 21-30 containing the HQDHTEDVE sequence) and the P5 peptide (SEQ ID NO:21 residues 27-36) reduced elastin binding by approximately 35% at the highest concentration tested, whereas the P3 peptide (SEQ ID NO:14 residues 14-23) inhibited binding of *S. aureus* to radiolabeled elastin by more than 95% in a concentration-dependent manner (Fig. 15). Sequence comparison of P3 and other active EbpS constructs revealed that the hexapeptide ^{18}TNSHQD^{23} (SEQ ID NO:16) is the only sequence shared by all members. However, the hexapeptide TNSHQD (P6) and its control TNSHQS (SEQ ID NO:17, P7) did not inhibit staphylococcal elastin binding at any concentration tested (0.075-2.00 mg/ml). These findings indicate that the region around the HQDHTEDVE sequence participates in elastin recognition.

Discussion

ECM adhesins are important for bacterial colonization of and dissemination through host tissues. The identification of the elastin binding site of EbpS is required for understanding the mechanism of *S. aureus* adhesion to elastin. Using overlapping EbpS fragments and recombinant constructs, the elastin binding site in EbpS was mapped to the amino terminal domain of the molecule. Overlapping synthetic peptides spanning amino acids 14-34 were then used to better define the binding domain. Among these, only peptides corresponding to residues 14-23 and 18-34 specifically inhibited elastin binding by more than 95%. Common to all active synthetic peptides and proteolytic and recombinant fragments of EbpS is the

hexameric sequence ^{18}Thr-Asn-Ser-His-Gln-Asp^{23}. Further evidence that this sequence is important for elastin binding was the loss of activity when Asp^{23} was substituted with Asn in the synthetic peptide corresponding to residues 18-34. However, the synthetic hexamer TNSHQD by itself did not inhibit staphylococcal binding to elastin. These findings indicate that although the presence of the TNSHQD sequence is essential for EbpS activity, flanking amino acids in the N- or C-terminal direction and the carboxyl side chain of Asp^{23} are required for elastin recognition.

The minimal requirements for elastin recognition by EbpS are unexpectedly similar to what has been observed for the interaction between *S. aureus* and fibronectin. Fibronectin binding to *S. aureus* is mediated by a surface fibronectin binding protein, and the fibronectin binding site in this adhesin has been mapped to an extracellular 38 amino acid motif repeated three times and partially a fourth time [Signäs, *et al.*, 1989, *supra*]. A subsequent investigation by McGavin *et al.* [*J. Biol. Chem.*, **266**:8343-8347 (1991)] has shown that essential amino acids are contained within residues 21-33 of the 38 amino acid motif and that flanking N- and C-terminal amino acids are required for activity. The carboxyl side chains of acidic amino acids are also essential. The flanking residues are required to acquire a conformation that is favorable for fibronectin binding.

Similar to the proposed ligand recognition mechanism for the staphylococcal fibronectin binding protein, the TNSHQD synthetic peptide by itself could be inactive because it folds improperly and flanking residues are required to form a secondary structure that is necessary for activity. The N-terminal region of full length EbpS is predicted to fold into amphipathic α helices except for regions including residues 14-23 which is where the TNSHQD sequence resides. These predictions indicate that although the TNSHQD sequence defines the elastin contact site, presentation of this region in an active manner requires flanking secondary sequences which would stabilize the structure of the elastin binding site in EbpS. This is one explanation for

why the binding affinities of active EbpS synthetic polypeptides for elastin are considerably lower than those of the larger elastin binding EbpS polypeptides.

The properties of staphylococcal elastin and fibronectin recognition mechanisms are opposite that of corresponding mammalian receptors. Mammalian receptors bind to their respective ligands through the interaction of structural domains in the receptor and a short contiguous peptide sequence in the ligand. Structural domains formed by both the α and β integrin subunits, for example, interact with short peptide sequences such as RGD [Pierschbacher, *et al., Nature (Lond.),* **309**:30-33 (1984)], LDV [Mould, *et al., J. Biol. Chem.*, **265**:4020-4024 (1990)], REDV [Mould, *et al., J. Biol. Chem.*, **266**:3579-3585 (1991)], and IDAPS [Mould, *et al., EMBO J.*, **10**:4089-4095 (1991)]. Similarly, the 67 kDa mammalian elastin binding protein recognizes the hydrophobic VGVAPG hexapeptide sequence [Hinek, *et al., Science,* **239**:1539-1541 (1988)]. In contrast, staphylococcal elastin and fibronectin adhesins interact with their ligands by fitting a small region of the adhesin into a structural binding pocket formed by the ligand. These differences in ligand recognition mechanisms can be important in both assuring a lack of binding competition between the staphylococcal and mammalian elastin and fibronectin binding systems, and in promoting efficient binding of staphylococci to host tissue components for colonization and dissemination purposes.

The different strategy in receptor-ligand recognition used by bacteria indicates that other elastin binding proteins may use a similar approach for interacting with elastin. Sequence comparisons, however, failed to detect the TNSHQD sequence in several known elastin-binding proteins, including pancreatic and neutrophil elastases [Fletcher, *et al., Biochemistry*, **26**:7256-7261 (1987); Shotton, *et al., Nature*, **225**:811-816 (1970); Steffensen, *et al., J. Biol. Chem.*, **270**:11555-11566 (1995)], lysostaphin [Park, *et al., Int. J. Biochem. Cell Biol.*, **27**:139-146 (1995)], microfibril-associated glycoprotein [Brown-Augsburger, *et al., J. Biol. Chem.*, **269**:28443-28449 (1994)], or lysozyme [Park, *et al., J. Invest. Dermatol.*, **106**:1075-1080 (1996)]. Furthermore,

none of these proteins contained significant homology to the extended EbpS sequence corresponding to residues 14-34.

The amino terminal third of the elastin protein is the sight of EbpS binding. An antibody generated against a peptide encoded by exons 9 and 10 of elastin specifically inhibits staphylococcal elastin binding indicating that the EbpS binding site is localized to this particular region.

Abbreviations as used herein,

EbpS: elastin binding protein of *Staphylococcus aureus*, ECM: extracellular matrix, MSCRAMMS: microbial surface components recognizing adhesive matrix molecules, rEbpS: full length recombinant EbpS, trEbpS: truncated recombinant EbpS, TBS: Tris-buffered saline, and TSB: tryptic soy broth.

Summary

A cell surface 25 kDa elastin binding protein of *Staphylococcus aureus* (EbpS) mediates binding of this pathogen to elastin. Results from binding assays examining the activity of fragments of EbpS suggested that residues 1-59 contain the elastin recognition site. Functional analysis of recombinant truncated forms of EbpS and synthetic peptides have been used to localize the elastin binding site to within a 21 amino acid region contained within residues 14-34 of the binding protein. Further evidence for the importance of this site was obtained by demonstrating that the inhibitory activity of anti-EbpS antibodies on staphylococcal elastin binding were neutralized when these antibodies were pre-absorbed with a truncated recombinant EbpS construct containing residues 1-34. Studies testing effects of overlapping synthetic peptides showed that sequences spanning amino acids Gln^{14}-Asp^{23} and Thr^{18}-Glu^{34} inhibit binding of *Staphylococcus aureus* to elastin. Substitution of Asp^{23} with Asn abrogated the blocking activity of the peptide, demonstrating the

requirement for a charged amino acid at this location. The composite data indicate that staphylococcal elastin binding is mediated by a discrete domain defined by short peptide sequences in the N-terminal extracellular region of EbpS.

While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

The following is a list of documents related to the above disclosure and particularly to the experimental procedures and discussions. The documents should be considered as incorporated by reference in their entirety.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Mecham, Robert P.
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(ii) TITLE OF INVENTION: BACTERIAL ELASTIN BINDING PROTEIN, NUCLEIC ACID SEQUENCE ENCODING SAME AND DIAGNOSTIC AND THERAPEUTIC MEHTODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 23

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(C) REFERENCE/DOCKET NUMBER: 1113-1-003 CIP

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 789 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 136..744

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AATTGACAAG CTATAATTAG TGTAATACAC AATTGAAAAA TGATTGAAAT AATTTGGAAA      60

AATATACCAT AAACATATGT CATGTGGGTA TATTTTATGT AAAATCATTG TAATAGAATA     120

GAAAGGAAGA TGGCT ATG TCT AAT AAT TTT AAA GAT GAC TTT GAA AAA AAT       171
                 Met Ser Asn Asn Phe Lys Asp Asp Phe Glu Lys Asn
                   1               5                  10
CGT CAA TCG ATA GAC ACA AAT TCA CAT CAA GAC CAT ACG GAA GAT GTT       219
Arg Gln Ser Ile Asp Thr Asn Ser His Gln Asp His Thr Glu Asp Val
          15                  20                  25

GAA AAA GAC CAA TCA GAA TTA GAA CAT CAG GAT ACA ATA GAG AAT ACG       267
Glu Lys Asp Gln Ser Glu Leu Glu His Gln Asp Thr Ile Glu Asn Thr
      30                  35                  40

GAG CAA CAG TTT CCG CCA AGA AAT GCC CAA AGA AGA AAA AGA CGC CGT       315
Glu Gln Gln Phe Pro Pro Arg Asn Ala Gln Arg Arg Lys Arg Arg Arg
  45                  50                  55                  60

GAT TTA GCA ACG AAT CAT AAT AAA CAA GTT CAC AAT GAA TCA CAA ACA       363
Asp Leu Ala Thr Asn His Asn Lys Gln Val His Asn Glu Ser Gln Thr
                  65                  70                  75

TCT GAA GAC AAT GTT CAA AAT GAG GCT GGC ACA ATA GAT GAT CGT CAA       411
Ser Glu Asp Asn Val Gln Asn Glu Ala Gly Thr Ile Asp Asp Arg Gln
              80                  85                  90

GTC GAA TCA TCA CAC AGT ACT GAA AGT CAA GAA CCT AGC CAT CAA GAC       459
Val Glu Ser Ser His Ser Thr Glu Ser Gln Glu Pro Ser His Gln Asp
          95                  100                 105

AGT ACA CCT CAA CAT GAA GAG GGA TAT TAT AAT AAG AAT GCT TTT GCA       507
Ser Thr Pro Gln His Glu Glu Gly Tyr Tyr Asn Lys Asn Ala Phe Ala
     110                 115                 120

ATG GAT AAA TCA CAT CCA GAA CCA ATC GAA GAC AAT GAT AAA CAC GAG       555
Met Asp Lys Ser His Pro Glu Pro Ile Glu Asp Asn Asp Lys His Glu
 125                 130                 135                 140

ACT ATT AAA GAA GCA GAA AAT AAC ACT GAG CAT TCA ACA GTT TCT GAT       603
Thr Ile Lys Glu Ala Glu Asn Asn Thr Glu His Ser Thr Val Ser Asp
                 145                 150                 155

AAG AGT GAA GCT GAA CAA TCT CAG CAA CCT AAA CCA TAT TTT GCA ACA       651
Lys Ser Glu Ala Glu Gln Ser Gln Gln Pro Lys Pro Tyr Phe Ala Thr
             160                 165                 170

GGT GCT AAC CAA GCA AAT ACA TCC AAA GAT AAA CAT GAT GAT GTA ACT       699
Gly Ala Asn Gln Ala Asn Thr Ser Lys Asp Lys His Asp Asp Val Thr
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175 180 185

GTT AAG CAA GAC AAA GAT GAA TCT AAA GAT CAT CAT AAG TGG TAA 744
Val Lys Gln Asp Lys Asp Glu Ser Lys Asp His His Lys Trp
190 195 200

AAAAGGCGCA GCAATTGGTG CTGGAACAGC GGGTGTTGCA GGTGC 789

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 202 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Asn Asn Phe Lys Asp Asp Phe Glu Lys Asn Arg Gln Ser Ile
1 5 10 15

Asp Thr Asn Ser His Gln Asp His Thr Glu Asp Val Glu Lys Asp Gln
20 25 30

Ser Glu Leu Glu His Gln Asp Thr Ile Glu Asn Thr Glu Gln Gln Phe
35 40 45

Pro Pro Arg Asn Ala Gln Arg Arg Lys Arg Arg Arg Asp Leu Ala Thr
50 55 60

Asn His Asn Lys Gln Val His Asn Glu Ser Gln Thr Ser Glu Asp Asn
65 70 75 80

Val Gln Asn Glu Ala Gly Thr Ile Asp Asp Arg Gln Val Glu Ser Ser
85 90 95

His Ser Thr Glu Ser Gln Glu Pro Ser His Gln Asp Ser Thr Pro Gln
100 105 110

His Glu Glu Gly Tyr Tyr Asn Lys Asn Ala Phe Ala Met Asp Lys Ser
115 120 125

His Pro Glu Pro Ile Glu Asp Asn Asp Lys His Glu Thr Ile Lys Glu
130 135 140

Ala Glu Asn Asn Thr Glu His Ser Thr Val Ser Asp Lys Ser Glu Ala
145 150 155 160

Glu Gln Ser Gln Gln Pro Lys Pro Tyr Phe Ala Thr Gly Ala Asn Gln
165 170 175

Ala Asn Thr Ser Lys Asp Lys His Asp Asp Val Thr Val Lys Gln Asp
180 185 190

Lys Asp Glu Ser Lys Asp His His Lys Trp

195 200

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asn Asn Phe Lys Asp Asp Phe Glu Lys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGTGGATCCA TAGAAAGGAA GGTGGCTGTG 30

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCAAAGCTTG CTGTACCAGC ACCAATT 27

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Asn Asn Phe Lys Asp Asp Phe Glu Lys Asn Arg Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

UCUUUCCUCC 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Pro Xaa Thr Gly Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 234 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGTCTAATA ATTTTAAAGA TGACTTTGAA AAAAATCGTC AATCGATAGA CACAAATTCA 60

CATCAAGACC ATACGGAAGA TGTTGAAAAA GACCAATCAG AATTAGAACA TCAGGATACA 120

ATAGAGAATA CGGAGCAACA GTTTCCGCCA AGAAATGCCC AAAGAAGAAA AAGACGCCGT 180

GATTTAGCAA CGAATCATAA TAAACAAGTT CACAATGAAT CACAAACATC TGAA 234

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Asn Asn Phe Lys Asp Asp Phe Glu Lys Asn Arg Gln Ser Ile
1 5 10 15

Asp Thr Asn Ser His Gln Asp His Thr Glu Asp Val Glu Lys Asp Gln
20 25 30

Ser Glu Leu Glu His Gln Asp Thr Ile Glu Asn Thr Glu Gln Gln Phe
35 40 45

Pro Pro Arg Asn Ala Gln Arg Arg Lys Arg Arg Arg Asp Leu Ala Thr
50 55 60

Asn His Asn Lys Gln Val His Asn Glu Ser Gln Thr Ser Glu
65 70 75

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGTCTAATA ATTTTAAAGA TGACTTTGAA AAAAATCGTC AATCGATAGA CACAAATTCA 60

CATCAAGACC ATACGGAAGA TGTTGAAAAA GACCAATCAG AA 102

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ser Asn Asn Phe Lys Asp Asp Phe Glu Lys Asn Arg Gln Ser Ile
1 5 10 15

Asp Thr Asn Ser His Gln Asp His Thr Glu Asp Val Glu Lys Asp Gln
20 25 30

Ser Glu

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Ser Ile Asp Thr Asn Ser His Gln Asp His Thr Glu Asp Val Glu
1 5 10 15

Lys Asp Gln Ser Glu
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Ser Ile Asp Thr Asn Ser His Gln Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Ser Ile Asp Thr Asn Ser His Gln Asp His Thr Glu Asp Val Glu

1 5 10 15

Lys Asp Gln Ser Glu Leu Glu His Gln Asp Thr Ile Glu Asn Thr Glu
20 25 30

Gln Gln Phe Pro Pro Arg Asn Ala Gln Arg Arg Lys Arg Arg
35 40 45

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Thr Asn Ser His Gln Asp
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Asn Ser His Gln Ser
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Thr Asn Ser His Gln Asp His Thr Glu Asp Val Glu Lys Asp Gln Ser
1 5 10 15

Glu

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Thr Asn Ser His Gln Asn His Thr Pro Asp Val Gln Lys Asp Gln Ser
1 5 10 15

Glu

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

His Gln Asp His Thr Glu Asp Val Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Gln Asp His Thr Glu Asp Val Glu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Val Glu Lys Asp Gln Ser Glu Leu Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Gln Asp Thr Ile Glu Asn Thr Glu
1 5

WHAT IS CLAIMED IS:

1. A microbial surface component recognizing adhesive matrix molecules (MSCRAMM), comprising a material selected from the group consisting of a protein, active fragments thereof, agonists thereof, mimics thereof, and combinations thereof, said MSCRAMM having the following characteristics:

a) binding to elastin;

b) inhibited activity in the presence of SDS; and

c) enhanced activity in the presence of thiol reductants.

2. The MSCRAMM of Claim 1 which is a member of the family of elastin binding proteins.

3. The MSCRAMM of Claim 1 which is bacterial in origin.

4. The MSCRAMM of Claim 1 which is a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, and active fragments thereof.

5. The MSCRAMM of Claim 1 which is derived from mammalian cells.

6. The MSCRAMM of Claim 1 labeled with a detectable label.

7. The MSCRAMM of Claim 6 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

8. An antibody to a MSCRAMM, the MSCRAMM to which said antibody is raised having the following characteristics:

a) binding to elastin;

b) inhibited activity in the presence of SDS; and

c) enhanced activity in the presence of thiol reductants.

9. The antibody of Claim 8 which is selected from the group consisting of polyclonal antibodies, monoclonal antibodies and chimeric antibodies.

10. The antibody of Claim 8 which is a monoclonal antibody.

11. An immortal cell line that produces a monoclonal antibody according to Claim 10.

12. The antibody of Claim 8 labeled with a detectable label.

13. The antibody of Claim 12 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

14. A DNA sequence or degenerate variant thereof, which encodes a MSCRAMM, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequence of SEQ ID NO:1;

(B) DNA sequences that hybridize to the foregoing DNA sequence under standard hybridization conditions; and

(C) DNA sequences that code on expression for an amino acid sequence encoded by the foregoing DNA sequence.

15. A recombinant DNA molecule comprising a DNA sequence or degenerate variant thereof, which encodes a MSCRAMM, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequence of SEQ ID NO:1;

(B) DNA sequences that hybridize to the foregoing DNA sequence under standard hybridization conditions; and

(C) DNA sequences that code on expression for an amino acid sequence encoded by the foregoing DNA sequence.

16. The recombinant DNA molecule of either of Claims 14 or 15, wherein said DNA sequence is operatively linked to an expression control sequence.

17. The recombinant DNA molecule of Claim 16, wherein said expression control sequence is selected from the group consisting of the early or late promoters of SV40 or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase and the promoters of the yeast α-mating factors.

18. A probe capable of screening for the MSCRAMM in alternate species prepared from the DNA sequence of Claim 14, or from SEQ ID NO:3.

19. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence or degenerate variant thereof, which encodes a MSCRAMM, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequence of SEQ ID NO:1;

(B) DNA sequences that hybridize to the foregoing DNA sequence under standard hybridization conditions; and

(C) DNA sequences that code on expression for an amino acid sequence encoded by the foregoing DNA sequence;

wherein said DNA sequence is operatively linked to an expression control sequence.

20. The unicellular host of Claim 19 wherein the unicellular host is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts, CHO.

R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells, insect cells, and human cells in tissue culture.

21. A method for detecting the presence or activity of a MSCRAMM, said MSCRAMM having the following characteristics: binding to elastin; inhibited activity in the presence of SDS; and enhanced activity in the presence of thiol reductants, wherein said MSCRAMM is measured by:

A. contacting a biological sample from a mammal in which the presence or activity of said MSCRAMM is suspected with a binding partner of said MSCRAMM under conditions that allow binding of said MSCRAMM to said binding partner to occur; and

B. detecting whether binding has occurred between said MSCRAMM from said sample and the binding partner;

wherein the detection of binding indicates that presence or activity of said MSCRAMM in said sample.

22. A method for detecting the presence and activity of a polypeptide ligand associated with a given invasive stimulus in mammals comprising detecting the presence or activity of a MSCRAMM according to the method of Claim 21, wherein detection of the presence or activity of the MSCRAMM indicates the presence and activity of a polypeptide ligand associated with a given invasive stimulus in mammals.

23. The method of Claim 22 wherein said invasive stimulus is an infection.

24. The method of Claim 23 wherein said infection is bacterial in origin.

25. A method for detecting the binding sites for a MSCRAMM, said MSCRAMM having the following characteristics:

A) binding to elastin;

B) inhibited activity in the presence of SDS; and

C) enhanced activity in the presence of thiol reductants; said method comprising:

i. placing a labeled MSCRAMM sample in contact with a biological sample from a mammal in which binding sites for said MSCRAMM are suspected;

ii. examining said biological sample in binding studies for the presence of said labeled MSCRAMM;

wherein the presence of said labeled MSCRAMM indicates a binding site for a MSCRAMM.

26. A method of testing the ability of a drug or other entity to modulate the activity of a MSCRAMM which comprises

A. culturing a colony of test cells which has a receptor for the MSCRAMM in a growth medium containing the MSCRAMM;

B. adding the drug under test; and

C. measuring the reactivity of said MSCRAMM with the receptor on said colony of test cells.

wherein said MSCRAMM has the following characteristics:

i) binding to elastin;

ii) inhibited activity in the presence of SDS; and

iii) enhanced activity in the presence of thiol reductants.

27. An assay system for screening drugs and other agents for ability to modulate the production of a MSCRAMM, comprising:

A. culturing an observable cellular test colony inoculated with a drug or agent;

B. harvesting a supernatant from said cellular test colony; and

C. examining said supernatant for the presence of said MSCRAMM

wherein an increase or a decrease in a level of said MSCRAMM indicates the ability

of a drug to modulate the activity of said MSCRAMM, said MSCRAMM having the following characteristics:

i) binding to elastin;

ii) inhibited activity in the presence of SDS; and

iii) enhanced activity in the presence of thiol reductants.

28. A test kit for the demonstration of a MSCRAMM in a eukaryotic cellular sample, comprising:

A. a predetermined amount of a detectably labelled specific binding partner of a MSCRAMM, said MSCRAMM having the following characteristics: binding to elastin; inhibited activity in the presence of SDS; and enhanced activity in the presence of thiol reductants;

B. other reagents; and

C. directions for use of said kit.

29. A test kit for demonstrating the presence of a MSCRAMM in a eukaryotic cellular sample, comprising:

A. a predetermined amount of a MSCRAMM, said MSCRAMM having the following characteristics: binding to elastin; inhibited activity in the presence of SDS; and enhanced activity in the presence of thiol reductants;

B. a predetermined amount of a specific binding partner of said MSCRAMM;

C. other reagents; and

D. directions for use of said kit;

wherein either said MSCRAMM or said specific binding partner are detectably labelled.

30. The test kit of Claim 28 or 29 wherein said labeled immunochemically reactive component is selected from the group consisting of polyclonal antibodies to

the MSCRAMM, monoclonal antibodies to the MSCRAMM, fragments thereof, and mixtures thereof.

31. A method of preventing and/or treating cellular debilitations, derangements and/or dysfunctions and/or other disease states in mammals, comprising administering to a mammal a therapeutically effective amount of a material selected from the group consisting of a MSCRAMM, an agent capable of promoting the production and/or activity of said MSCRAMM, an agent capable of mimicking the activity of said MSCRAMM, an agent capable of inhibiting the production of said MSCRAMM, and mixtures thereof, or a specific binding partner thereto, said MSCRAMM having the following characteristics:

a) binding to elastin;

b) inhibited activity in the presence of SDS; and

c) enhanced activity in the presence of thiol reductants.

32. The method of Claim 31 wherein said disease states include those caused at least in part by bacterial infection.

33. The method of Claim 31 wherein said MSCRAMM is administered to modulate the course of therapy where MSCRAMM is being administered as the primary therapeutic agent.

34. The method of Claim 31 wherein said MSCRAMM is administered to modulate the course of therapy where MSCRAMM is being co-administered with one or more additional therapeutic agents.

35. A pharmaceutical composition for the treatment of cellular debilitation, derangement and/or dysfunction in mammals, comprising:

A. a therapeutically effective amount of a material selected from the group consisting of a MSCRAMM, an agent capable of promoting the production

and/or activity of said MSCRAMM, an agent capable of mimicking the activity of said MSCRAMM. an agent capable of inhibiting the production of said MSCRAMM, and mixtures thereof, or a specific binding partner thereto, said MSCRAMM having the following characteristics: binding to elastin; inhibited activity in the presence of SDS; and enhanced activity in the presence of thiol reductants; and

B. a pharmaceutically acceptable carrier.

36. The MSCRAMM of Claim 1, wherein said MSCRAMM has a predicted molecular weight of about 25 KD, and a predicted PI of about 4.9.

37. The MSCRAMM of Claim 3, wherein said MSCRAMM is isolated from *Staphylococcus aureus*.

38. A polypeptide comprising an amino acid sequence that corresponds to the elastin binding site of a microbial surface component recognizing adhesive matrix molecules (MSCRAMM), wherein said polypeptide consists of between 8 and 80 amino acids, and binds elastin.

39. The polypeptide of Claim 38, wherein the MSCRAMM has the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:2 comprising a conservative substitution thereof.

40. The polypeptide of Claim 38 having the amino acid sequence consisting of the N-terminal 59 amino acids of the MSCRAMM.

41. The polypeptide of Claim 38 that further inhibits the binding of *S. aureus* to elastin.

42. A pharmaceutical composition comprising the polypeptide of Claim 41 and a pharmaceutically acceptable carrier.

43. A method of treating a Staphylococcus aureus infection by administering a therapeutically effective amount of the pharmaceutical composition of Claim 42.

44. The polypeptide of Claim 38 in which said elastin binding site comprises the amino acid sequence of SEQ ID NO:16 or SEQ ID NO:16 comprising a conservative substitution thereof .

45. The polypeptide of Claim 44 that has the amino acid sequence of SEQ ID NO:10 or SEQ ID NO:10 comprising a conservative substitution thereof.

46. The polypeptide of Claim 44 that contains between 10 and 46 amino acids.

47. The polypeptide of Claim 46 comprising the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:14 comprising a conservative substitution thereof.

48. The polypeptide of Claim 46 that contains about 10 amino acids.

49. The polypeptide of Claim 46 comprising the amino acid sequence of SEQ ID NO:18 or SEQ ID NO:18 comprising a conservative substitution thereof.

50. The polypeptide of Claim 49 that has the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:12 comprising a conservative substitution thereof.

51. An immunogenic composition comprising the polypeptide of Claim 38 in an admixture with an adjuvant.

52. The immunogenic composition of Claim 51 wherein the polypeptide is conjugated to a carrier molecule.

53. A method for generating an antibody to the elastin-binding domain of a MSCRAMM comprising immunizing an animal with the composition of Claim 51.

54. The antibody of Claim 53 which is selected from the group consisting of a polyclonal antibody and a monoclonal antibody.

55. A nucleic acid encoding a polypeptide that is between 8 and 80 amino acids, wherein said polypeptide binds elastin and comprises an amino acid sequence that corresponds to the elastin binding site of a microbial surface component recognizing adhesive matrix molecules (MSCRAMM).

56. The nucleic acid of Claim 55 having the nucleic acid sequence of SEQ ID NO:9.

57. The nucleic acid of Claim 55 which is DNA.

58. A cloning vector which comprises the DNA of Claim 57.

59. An expression vector which comprises the DNA of Claim 57 operatively associated with an expression control sequence.

60. A unicellular host transfected or transformed with the expression vector of Claim 59.

61. The unicellular host of Claim 60 that is a bacterium.

62. A mammalian cell transfected or transformed with the expression vector of Claim 59.

63. A method of expressing the polypeptide of Claim 38 comprising culturing a cell containing an expression vector comprising a nucleic acid encoding the polypeptide under conditions that allow the polypeptide to be expressed.

64. The method of Claim 63 further comprising the step of purifying the expressed polypeptide.

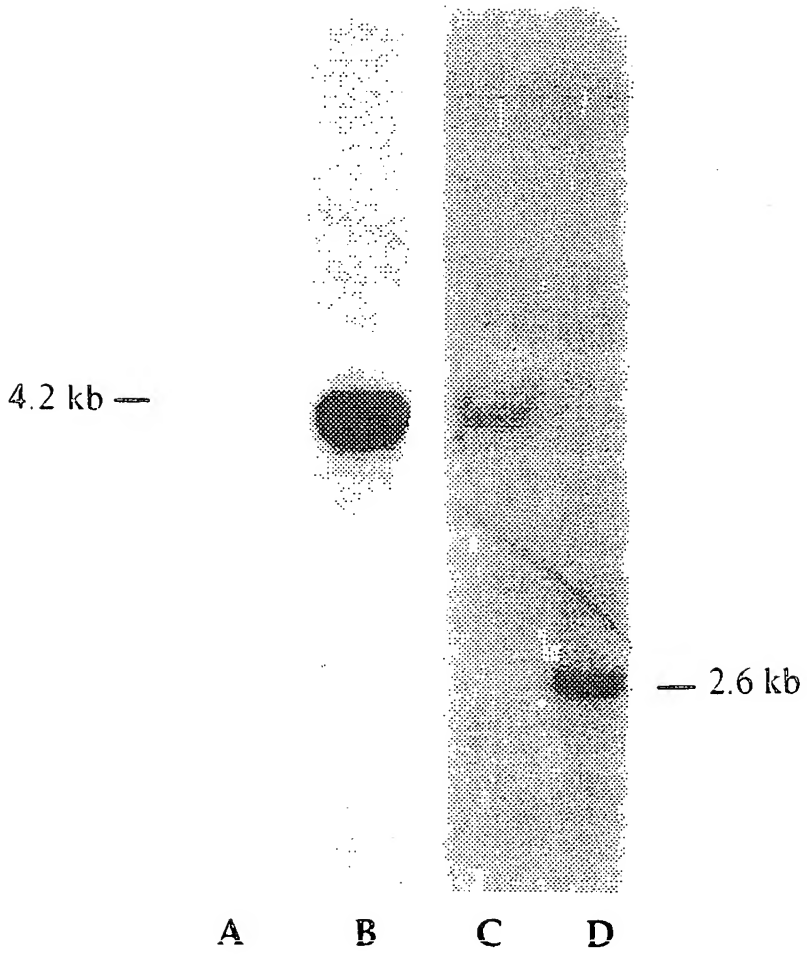

FIG. 1

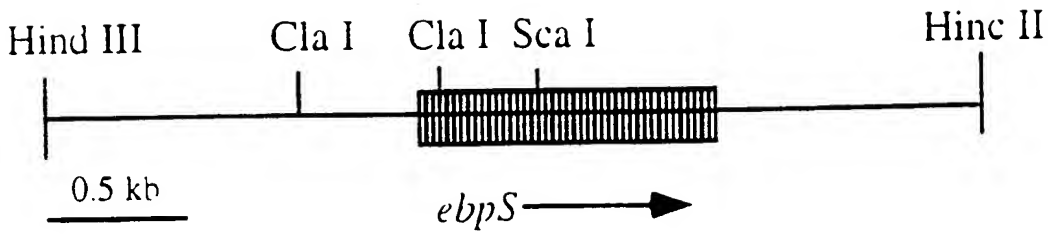

FIG.2

```
-135  AATTGACAAGCTATAATTAGTGTAATACACAATTGAAAAATGATTGAAATAATT

-81   TGGAAAAATATACCATAAACATAIGTCATGTGGGTATATTTTATGTAAAAICAT
                           -35                    -10

-27   TGTAATAGAATAGAAAGGAAGATGGCTATGTCTAATAATTTTAAAGATGACTTT
 1                  RbS          Met Ser Asn Asn Phe Lys Asp Asp Phe

 28   GAAAAAAATCGTCAATCGATAGACACAAATTCACATCAAGACCATACGGAAGAT
 10   Glu Lys Asn Arg Gln Ser Ile Glu Thr Asn Ser His Gln Asp His Thr Glu Asp

 82   GTTGAAAAAGACCAATCAGAATTAGAACATCAGGATACAATAGAGAATACGGAG
 28   Val Glu Lys Asp Gln Ser Glu Leu Glu His Gln Asp Thr Ile Glu Asn Thr Glu

136   CAACAGTTTCCGCCAAGAAATGCCCAAAGAAGAAAAAGACGCCGTGATTTAGCA
 46   Gln Gln Phe Pro Pro Arg Asn Ala Gln Arg Arg Lys Arg Arg Arg Asp Leu Ala

190   ACGAATCATAATAAACAAGTTCACAATGAATCACAAACATCTGAAGACAATGTT
 64   Thr Asn His Asn Lys Gln Val His Asn Glu Ser Gln Thr Ser Glu Asp Asn Val

244   CAAAATGAGGCTGGCACAATAGATGATCGTCAAGTCGAATCATCACACAGTACT
 82   Gln Asn Glu Ala Gly Thr Ile Asp Asp Arg Gln Val Glu Ser Ser His Ser Thr

298   GAAAGTCAAGAACCTAGCCATCAAGACAGTACACCTCAACATGAAGAGGGATAT
100   Glu Ser Gln Glu Pro Ser His Gln Asp Ser Thr Pro Gln His Glu Glu Gly Tyr

352   TATAATAAGAATGCTTTTGCAATGGATAAATCACATCCAGAACCAATCGAAGAC
118   Tyr Asn Lys Asn Ala Phe Ala Met Asp Lys Ser His Pro Glu Pro Ile Glu Asp

406   AATGATAAACACGAGACTATTAAAGAAGCAGAAAATAACACTGAGCATTCAACA
136   Asn Asp Lys His Glu Thr Ile Lys Glu Ala Glu Asn Asn Thr Glu His Ser Thr

460   GTTTCTGATAAGAGTGAAGCTGAACAATCTCAGCAACCTAAACCATATTTTGCA
154   Val Ser Asp Lys Ser Glu Ala Glu Gln Ser Gln Gln Pro Lys Pro Tyr Phe Ala

514   ACAGGTGCTAACCAAGCAAATACATCCAAAGATAAACATGATGATGTAACTGTT
172   Thr Gly Ala Asn Gln Ala Asn Thr Ser Lys Asp Lys His Asp Asp Val Thr Val

568   AAGCAAGACAAAGATGAATCTAAAGATCATCATAAGTGGTAAAAAAGGCGCAGC
190   Lys Gln Asp Lys Asp Glu Ser Lys Asp His His Lys Trp  •

622   AATTGGTGCTGGAACAGCGGGTGTTGCAGGTGC
```

FIG.3

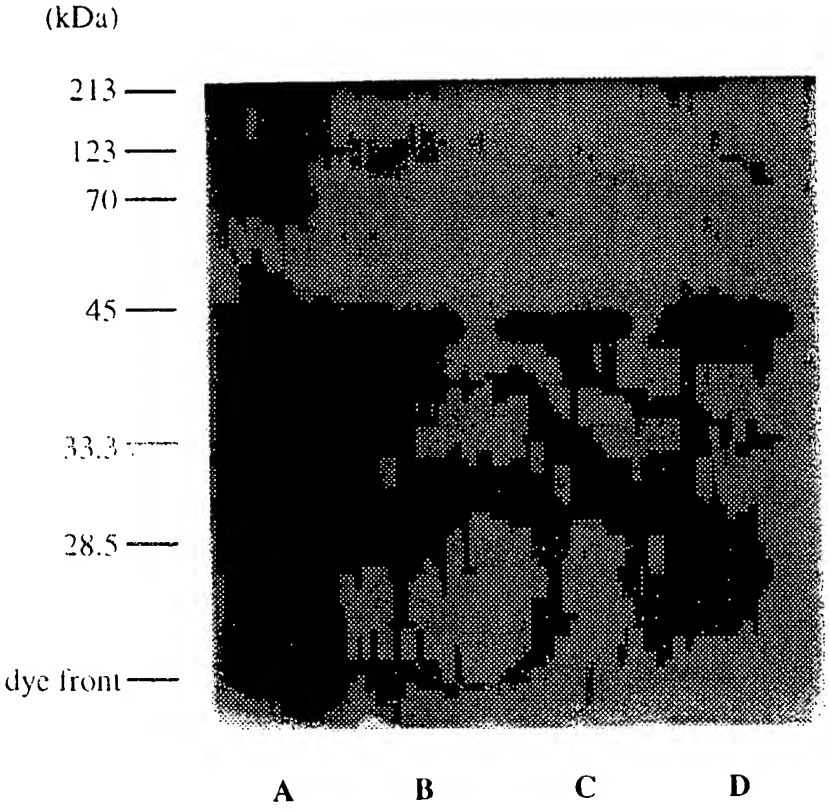

FIG.4

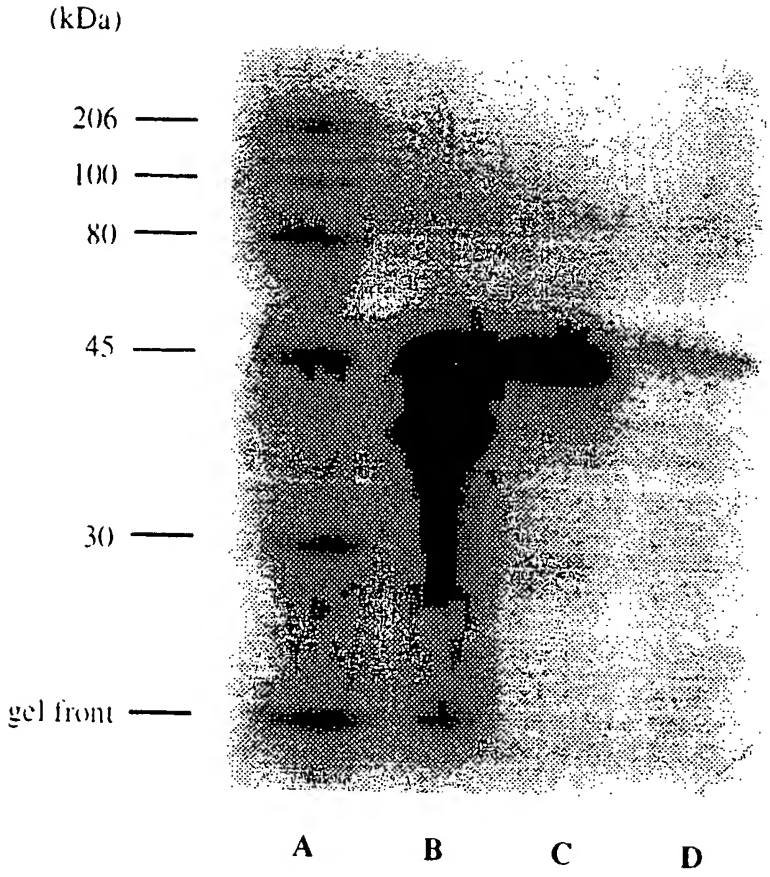

FIG.5

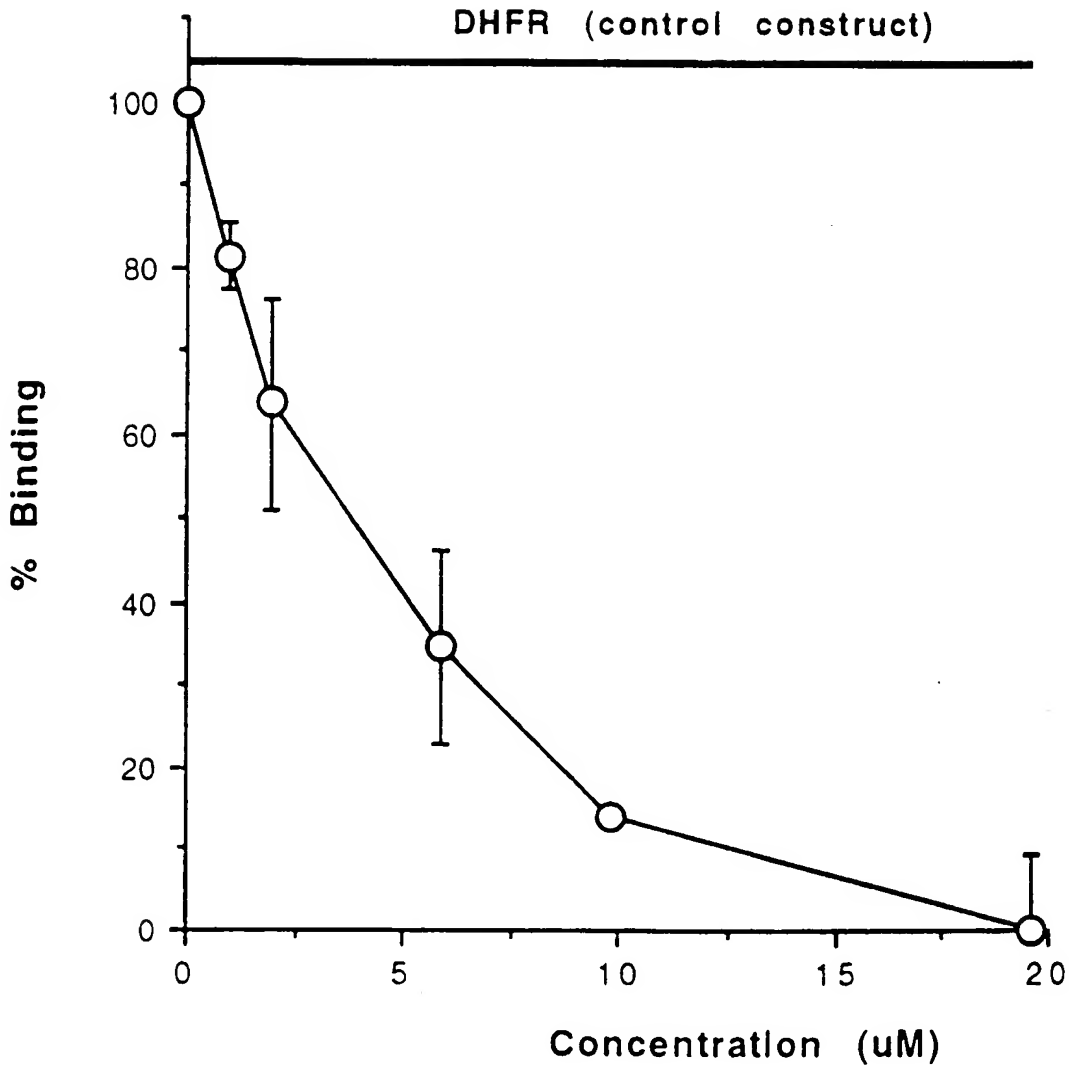
DHFR (control construct)
100
80
60
40
20
0
% Binding
0
5
10
15
20
Concentration (uM)

FIG.6

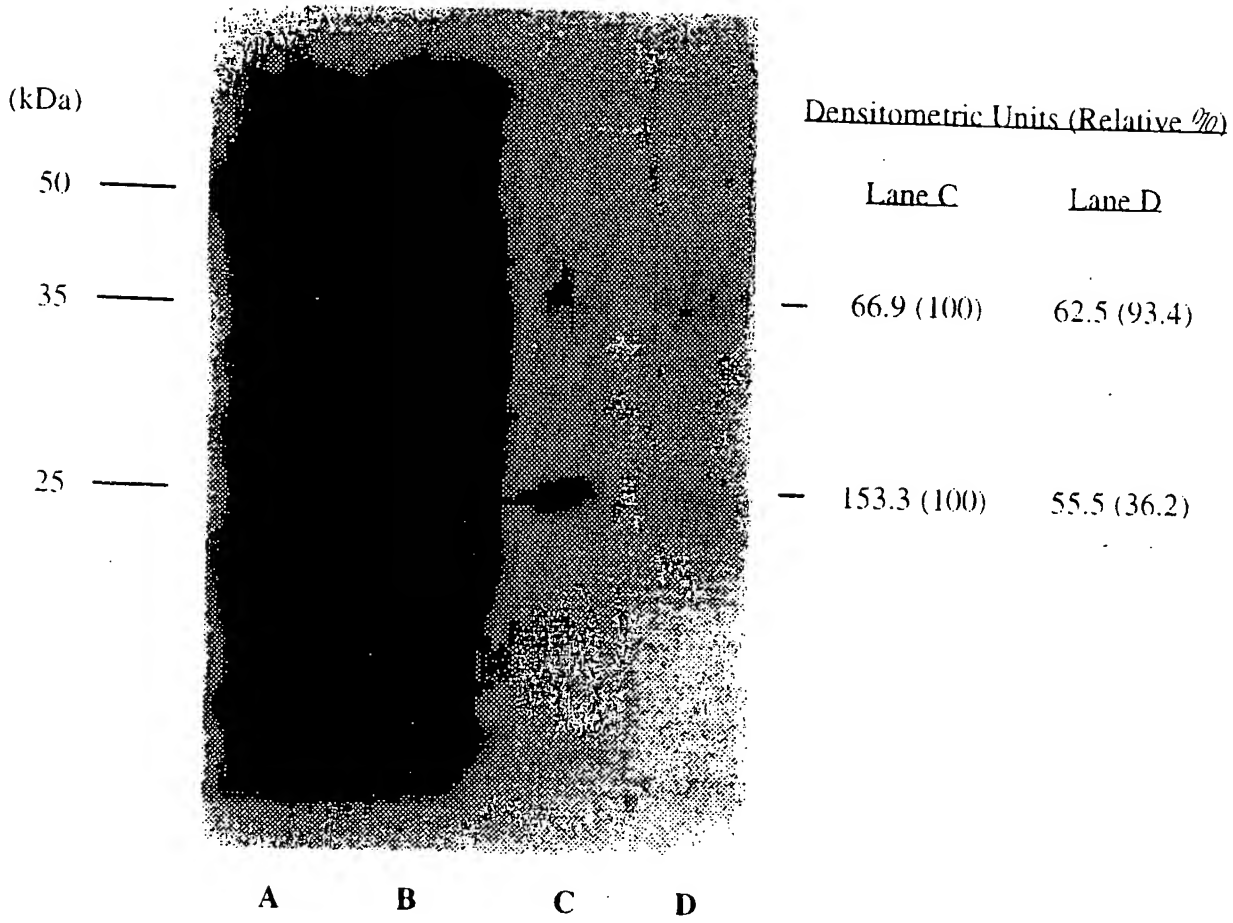
(kDa)
50
35
25
A
B
C
D
Densitometric Units (Relative %)
Lane C
Lane D
66.9 (100)
62.5 (93.4)
153.3 (100)
55.5 (36.2)

FIG.7

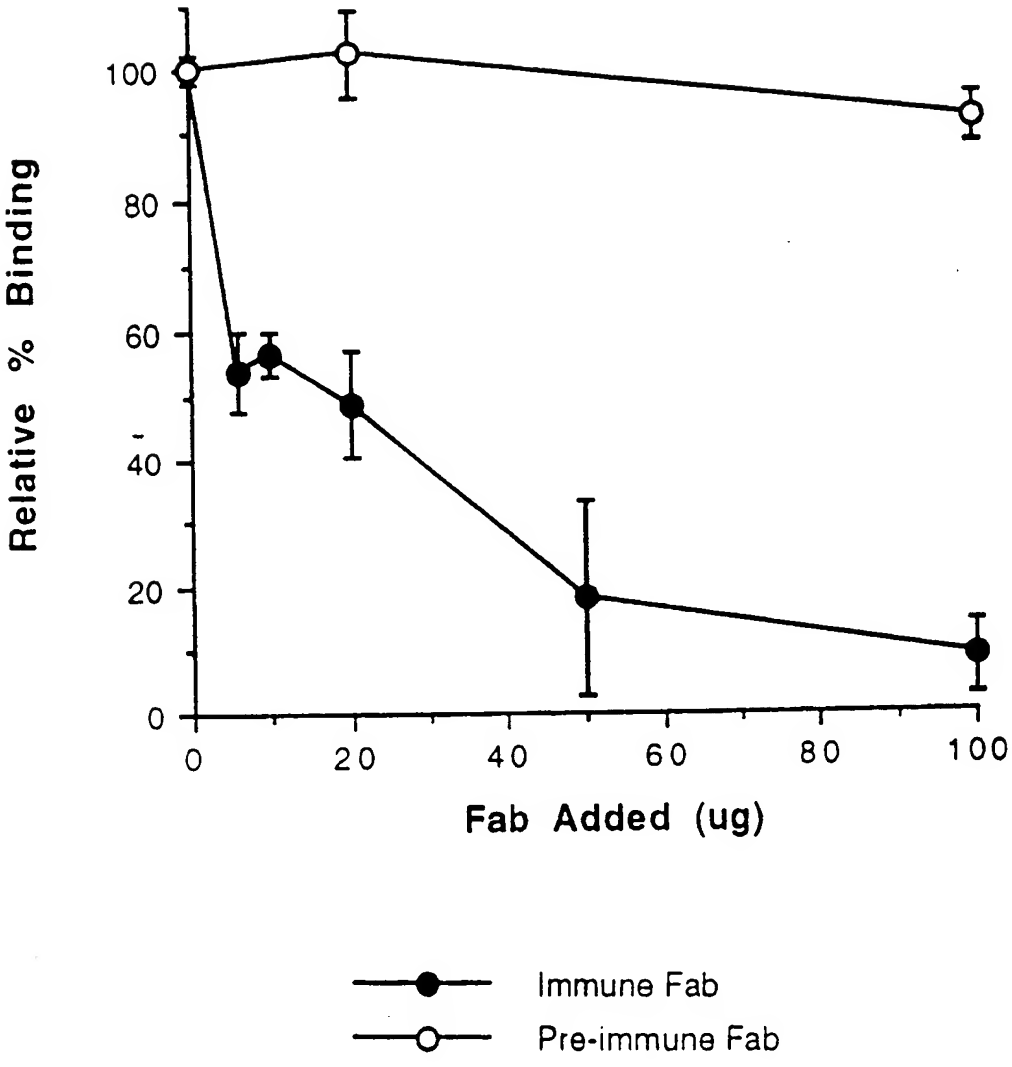
Relative % Binding
100
80
60
40
20
0
0
20
40
60
80
100
Fab Added (ug)
Immune Fab
Pre-immune Fab

FIG.8

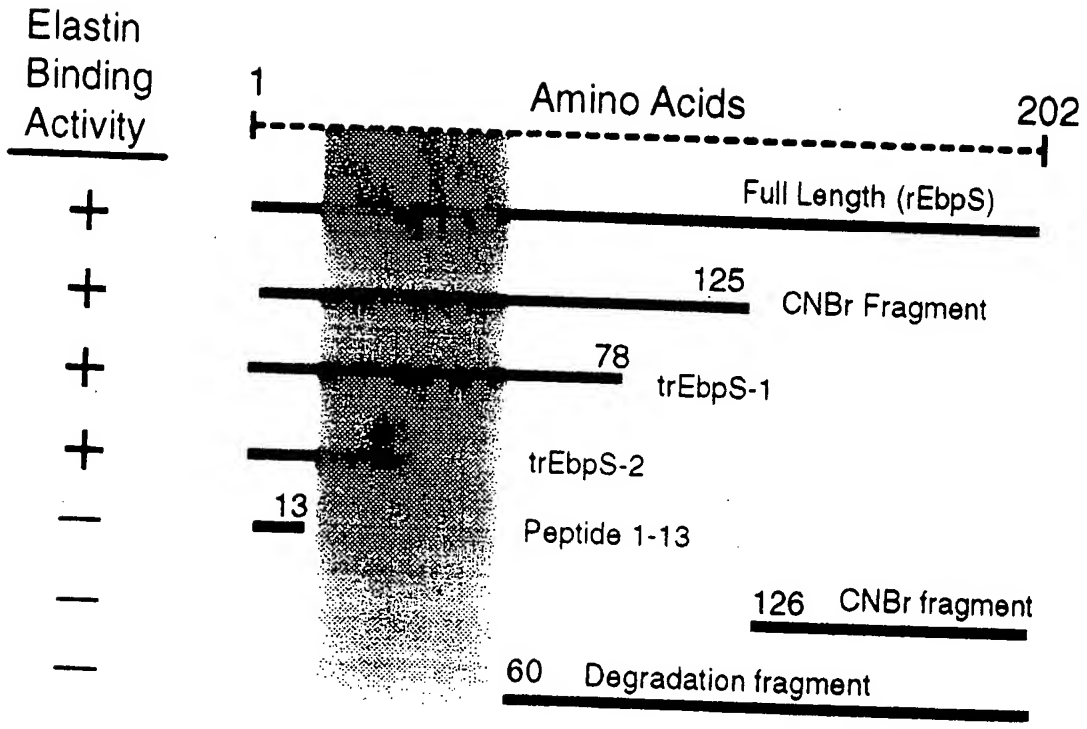
Elastin
Binding
Activity
1
Amino Acids
202
Full Length (rEbpS)
125
CNBr Fragment
78
trEbpS-1
trEbpS-2
13
Peptide 1-13
126 CNBr fragment
60 Degradation fragment
+
+
+
+
—
—
—

FIG.9

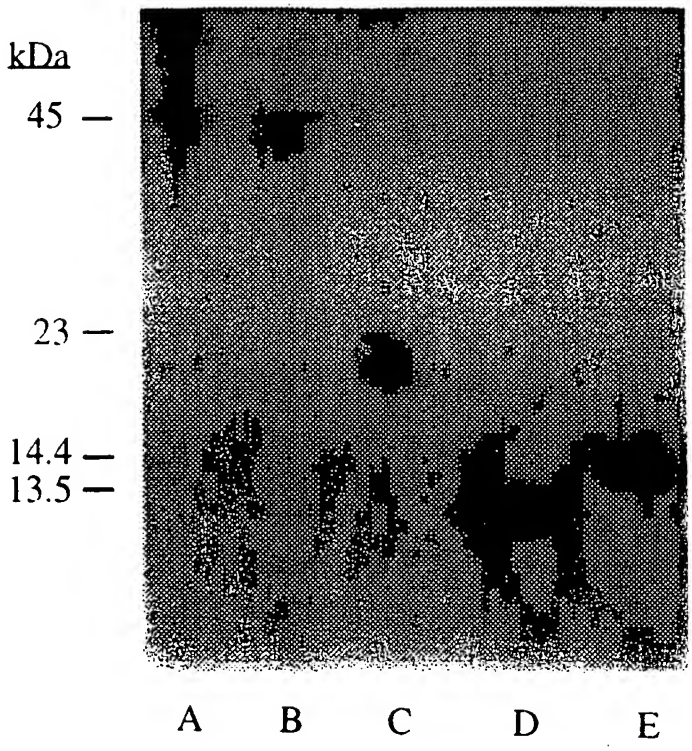

FIG.10A

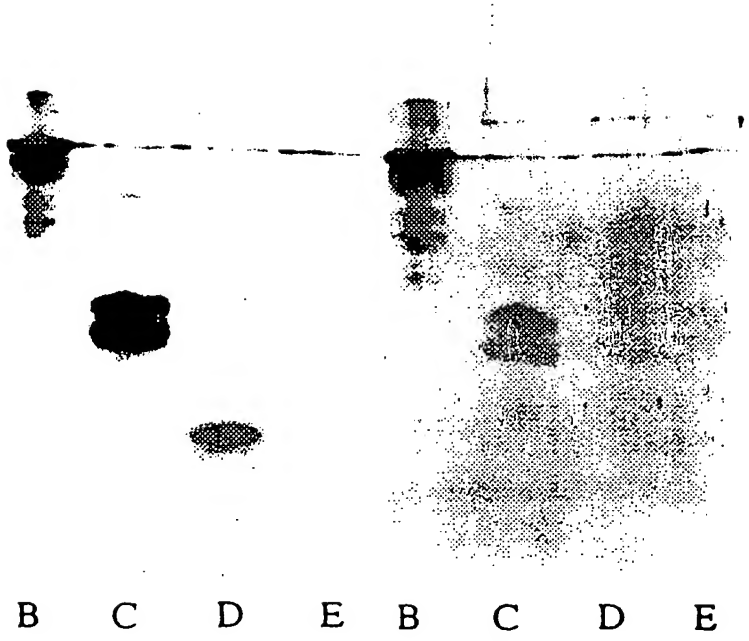

FIG.10B FIG.10C

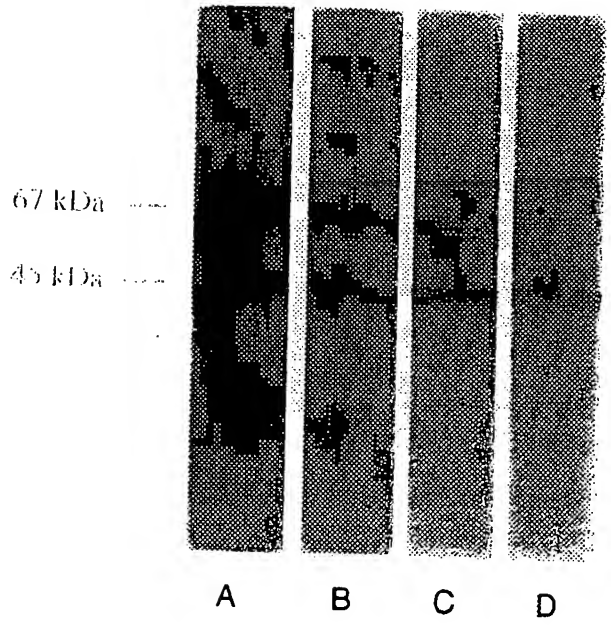

FIG.11

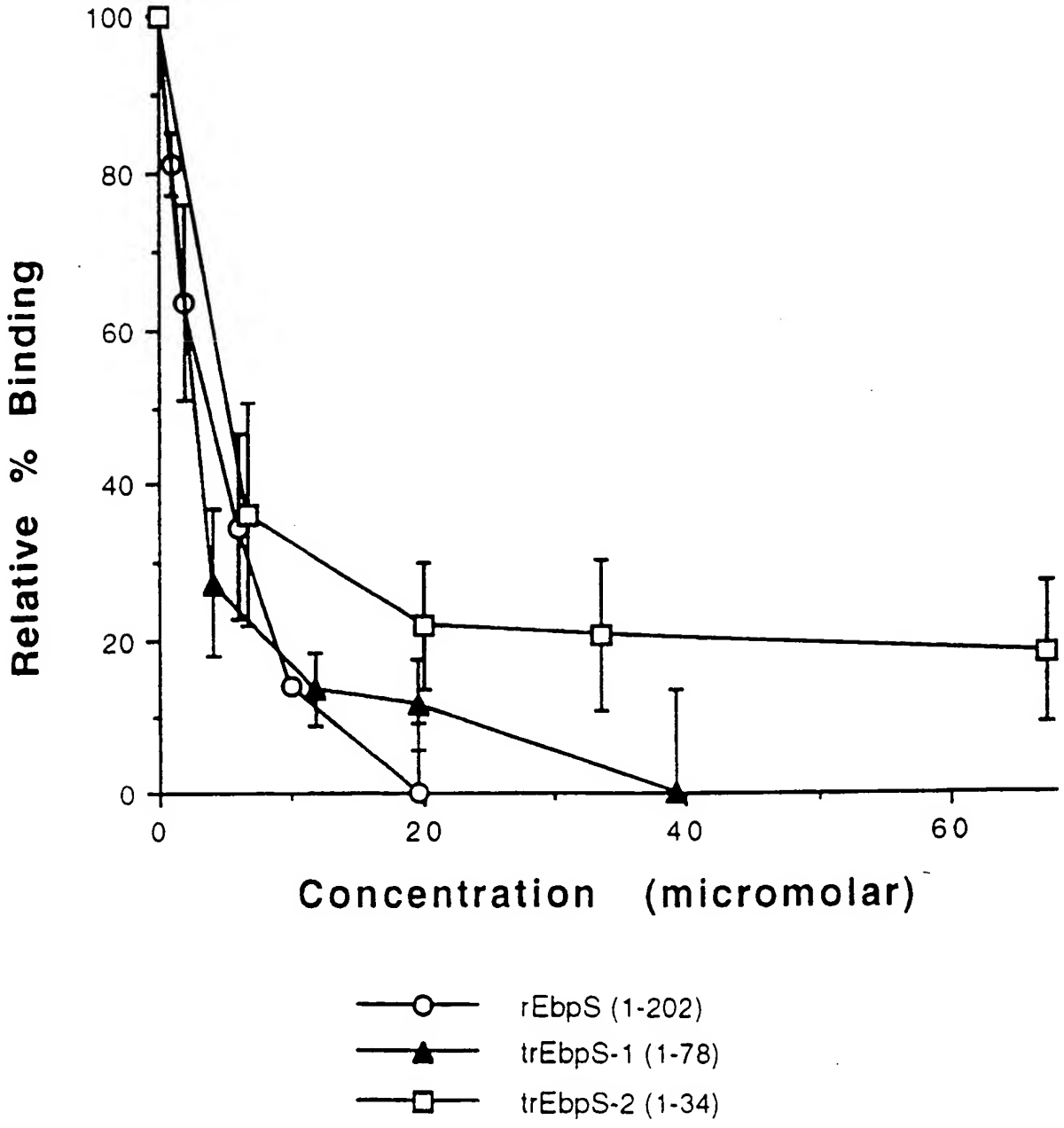
Relative % Binding
100
80
60
40
20
0
0
20
40
60
Concentration (micromolar)
rEbpS (1-202)
trEbpS-1 (1-78)
trEbpS-2 (1-34)

FIG. 12

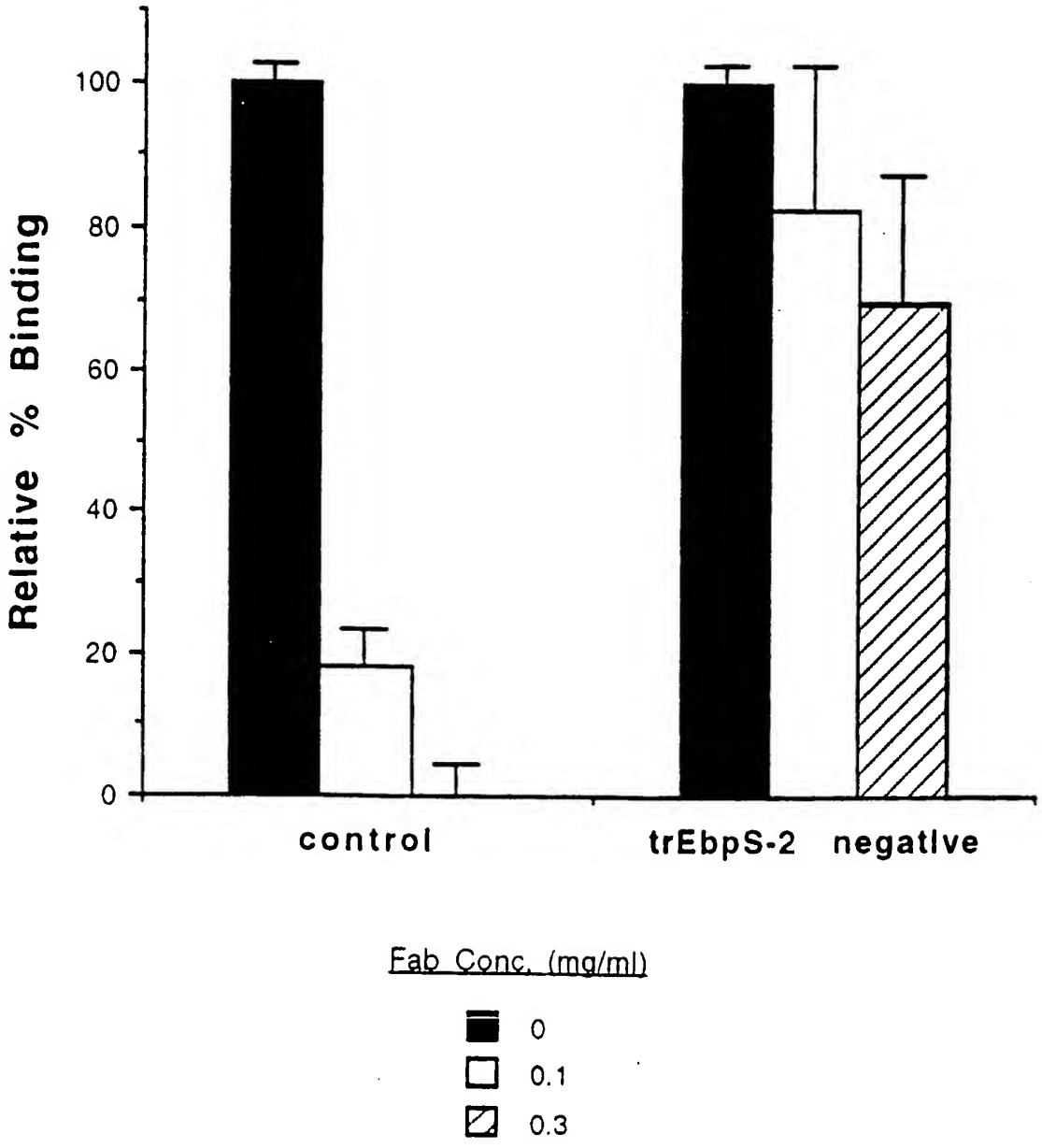
Relative % Binding
100
80
60
40
20
0
control
trEbpS-2 negative
Fab Conc. (mg/ml)
0
0.1
0.3

FIG. 13

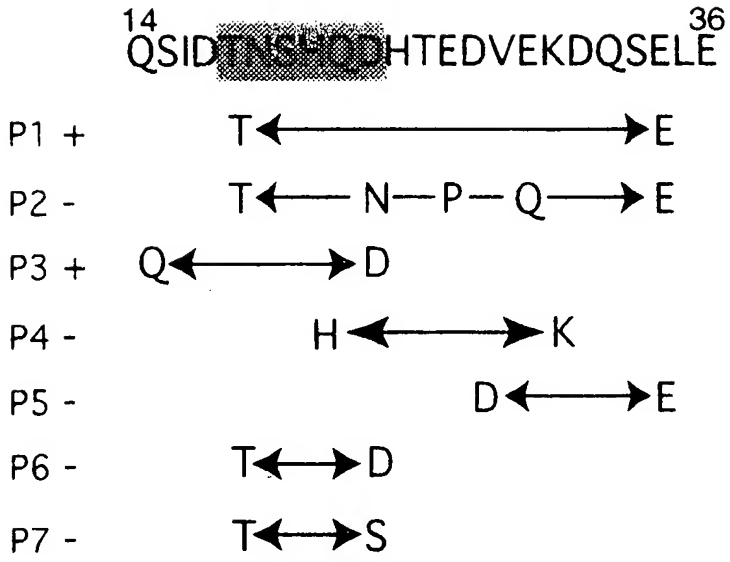

FIG.14

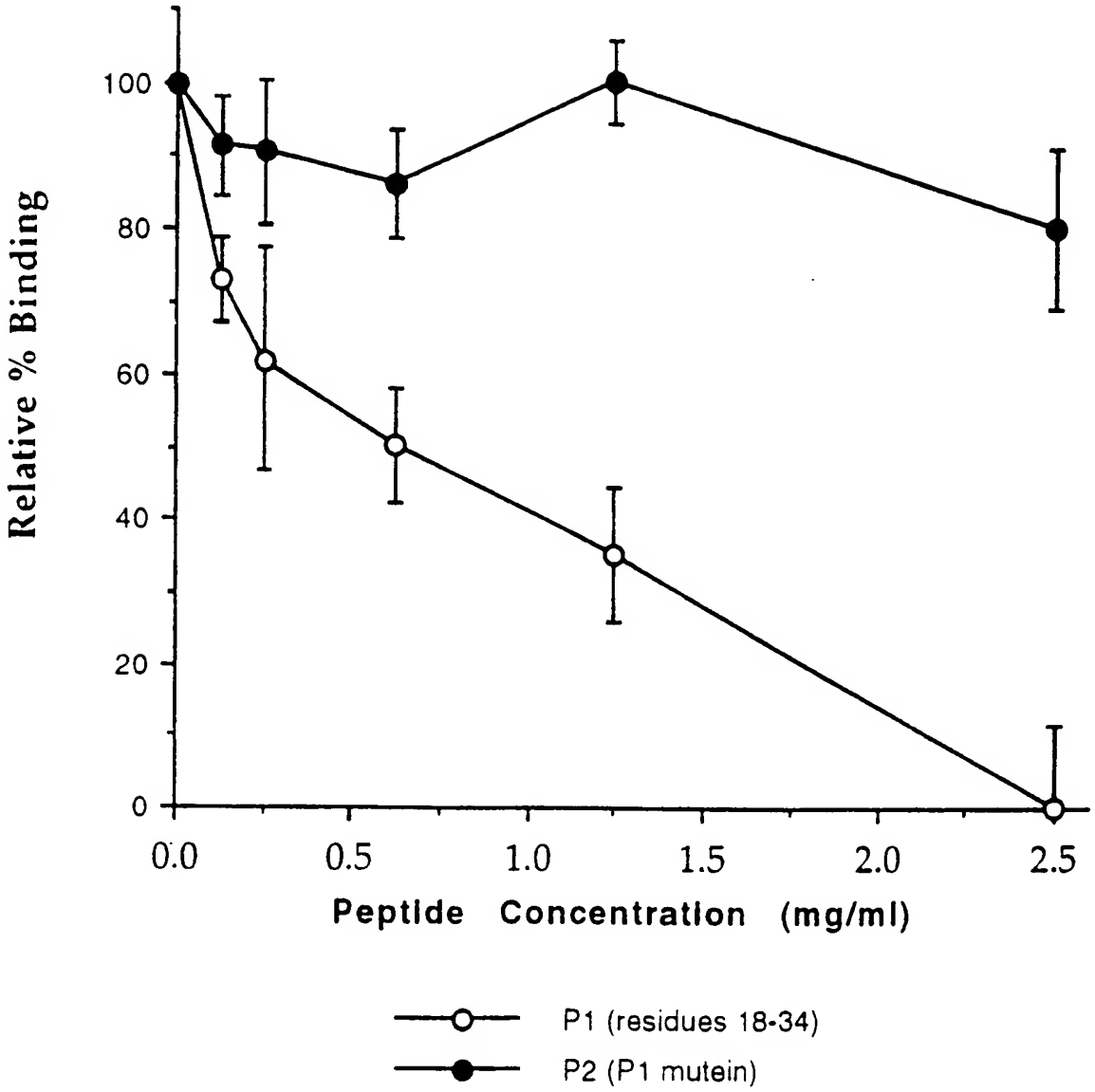
Relative % Binding
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
Peptide Concentration (mg/ml)
P1 (residues 18-34)
P2 (P1 mutein)

FIG.15

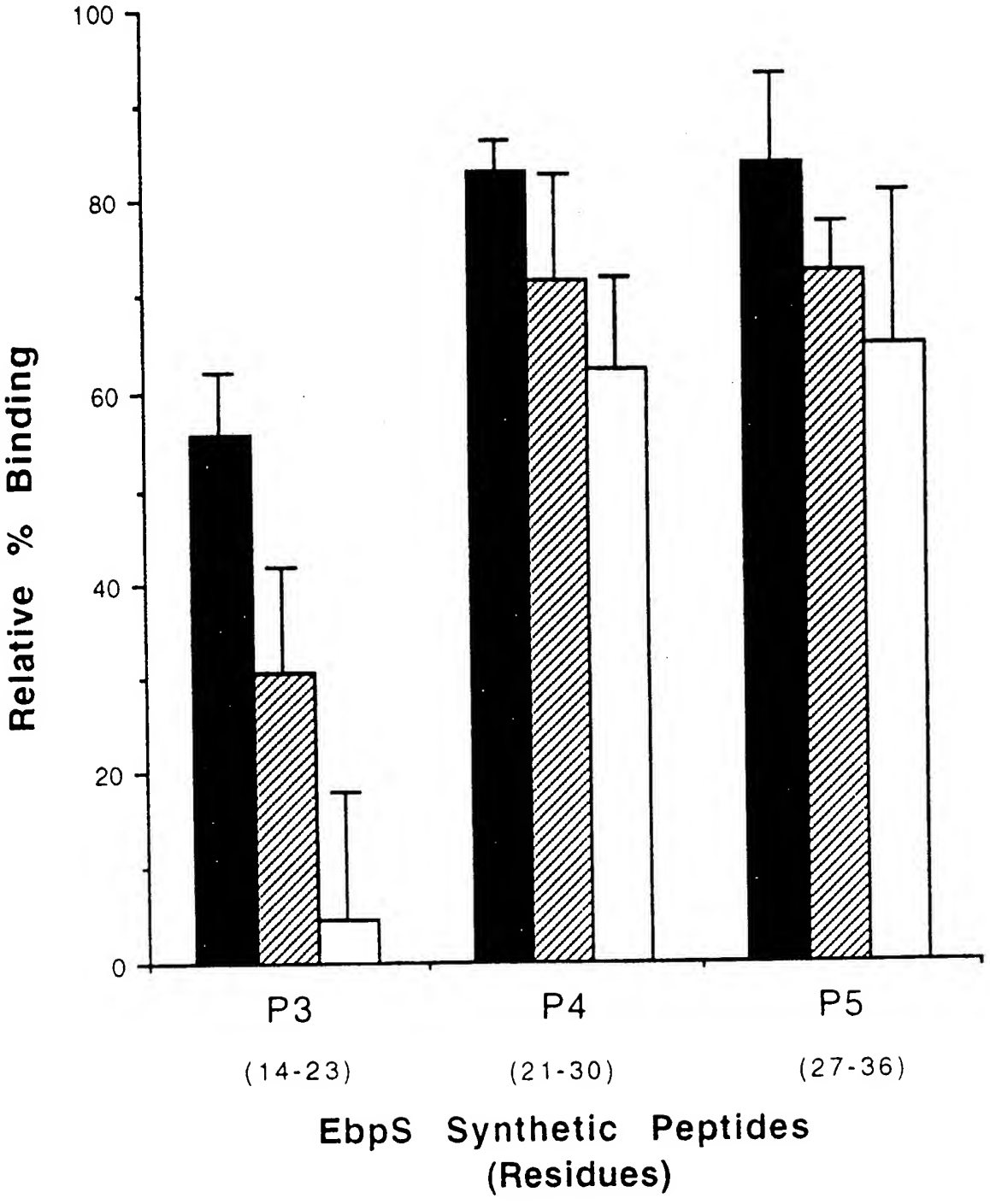
100
80
60
40
20
0
Relative % Binding
P3
(14-23)
P4
(21-30)
P5
(27-36)
EbpS Synthetic Peptides
(Residues)
0.5 mg/ml
1.0 mg/ml
2.0 mg/ml

FIG.16

INTERNATIONAL SEARCH REPORT

Int. nal Application No
PCT/US 97/03106

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C07K14/31 G01N33/50 C07K16/12 A61K39/085
C12N5/12 C12Q1/68 C12N1/21 C12N1/19 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	J BIOL CHEM, JUN 28 1996, 271 (26) P15803-9, UNITED STATES, XP002034585 PARK PW ET AL: "Molecular cloning and expression of the gene for elastin-binding protein (ebpS) in Staphylococcus aureus." see the whole document	1-64
P,X	& PIR Databes entry TRPRO Accession number Q53630; 01 Nov 1996 PARK ET AL: 'Elastin binding protein' see abstract	1-64

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 1997	23.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,	Espen ...

INTERNATIONAL SEARCH REPORT

Inte. nal Application No
PCT/US 97/03106

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEMISTRY, APR 2 1991, 30 (13) P3346-50, UNITED STATES, XP002034586 GROSSO LE ET AL: "Characterization of a putative clone for the 67-kilodalton elastin/laminin receptor suggests that it encodes a cytoplasmic protein rather than a cell surface receptor." see figures 1,4,5 ---	1,2,5-9
X	J BIOL CHEM, DEC 5 1991, 266 (34) P23399-406, UNITED STATES, XP002034587 PARK PW ET AL: "Binding of elastin to Staphylococcus aureus." cited in the application see figures 10,11; table II ---	1-13, 36-41, 44-50
X	MOLECULAR BIOLOGY OF THE CELL, 5 (SUPPL.). 1994. 59A., XP002034588 PARK P W ET AL: "Cloning, sequencing, and expression of the staphylococcal elastin binding protein" see abstract -----	1-64